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NUCLEAR FORM AS RELATED TO FUNCTIONAL ACTIVITIES OF NORMAL AND PATHOLOGICAL CELLS

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(WITH ELEVEN FIGURES)

Introduction

There is considerable evidence that the relations of the nucleus to metabolic activities involve its assumption of irregular forms, which have been generally interpreted as having their significance in the resulting increase of nuclear surface in relation to the cytoplasm. The nuclei of cells infected by fungi frequently are found to be hypertrophied, amoeboid, or lobed in outline, and this is regarded as direct evidence of pathological changes in the cell metabolism. Perhaps the most outstanding and fully studied case of abnormal nuclear form, either in plant or animal literature, is that of the nuclei of the giant cancer cells, which are extraordinarily hypertrophied, exceedingly irregular and deeply lobed, and have been said to multiply amitotically, by budding or by fragmentation.

While making a cytological study of certain variegated plants, in which this condition is assumed not to be induced by viruses, the writer fixed and sectioned for study material from *Lilium longiflorum* and its variegated form *L. longiflorum foliis albomarginatis*. Lily tissues are well known as favorable material for cytological study, and in certain cells in various parts of the plants very irregular nuclear forms were found.

Other material from both healthy and diseased lily plants was fixed and sectioned for study, and a wide range of nuclear types was found. These types included giant hypertrophied rounded nuclei; giant and minute so-called amoeboid nuclei; giant lobed or lobulate nuclei; nuclei with surfaces projecting outward as pointed teeth, horns, and rounded knobs; nuclei with so-called canals and furrows; nuclei apparently dividing by budding and constriction, and in all stages of apparent fragmentation, chromatin degeneration, and shrinkage. The tissues of some 70 different specimens of lily plants, including 20 varieties, have been studied. The material was obtained at the New York Botanical Gardens, R. A. HARPER'S farm at Ridgewood, New Jersey, the florist shops of the city, and the greenhouses at Columbia University. In a preliminary way plants have been classed as healthy if they were of normal size, with a strong green leaf coloring, if they bore flowers and well developed fruits, and showed no perceptible yellowing or streaking of the leaves. Leaves turning yellow at the base of the plant were not necessarily considered as indicating a pathological condition. Plants suspected of being affected with mosaic because of the presence of light green or yellow green areas or streaks in the leaves were classed as pathological. Other pathological material was taken from plants which were stunted, dwarfed, or showed yellowing, whether due to poor cultural conditions or a specific pathogen.

For a study of the nucleus and its relation to metabolic activity in storage organs, apparently healthy material was used from the tubers of dahlia; the bulbs of hyacinth and narcissus; the cotyledons of sprouting soy beans, bush beans, and peas; and the endosperm and embryo of buckwheat, oat, corn, and castor bean. The material was fixed with either Flemming's medium strong solution, or with Allen's B 15 solution. The latter fixative gave most excellent results, especially for the nuclei. Sections were cut at 5μ or 7.5μ and stained with Flemming's triple stains.

Tables I and II summarize data from the literature as to the apparent relation of tissue differentiation to nuclear form. I have included nuclei said to be dividing amitotically with other cases of lobed or constricted types.

TABLE I

OUTLINE OF DATA RELATING METABOLIC ACTIVITY TO NUCLEAR FORM

AUTHOR	DATE	PLANT OR ANIMAL STUDIED	TISSUE OR ORGAN CONCERNED	NUCLEAR FORM
Korschelt	1884	Chironomus	Salivary glands	Irregular, lobed, amoeboid, toothed?
Howell	1889	Insects	Egg nurse cells	Amoeboid
	1890	Young kitten	Developing bone	Lobed, fragmenting
Müller	1892	Man	Cancer cells	Irregular, fragmenting, degenerating
Korschelt	1896	Insects	Spinning glands	Branched network
		Crustacea	Salivary glands	Branched network
Foot	1897	Animal	Yolk nucleus	Amoeboid
Schniewind-Thies	1897	Monocotyledonous plants	Nectaries	Lobed, incised, branched
Molisch	1899	Musa chinensis	Latex ducts	"Blasenkerne"
		Lycoris radiata	Slime ducts	"Fadenkerne"
		Aloe	Secretory cells	Giant, lobulate
Torrey	1902	Zea mays, other grasses	Epidermal layer of the scutellum	Markedly lobed
Bashford and Murray	1904	Cat, mouse	Carcinoma, epithelioma	Amoeboid, amitotic
Reed	1904	Zea mays	Epidermal cells of scutellum	Distorted, swollen
Unna	1905	Man	Carcinoma	Lobed, fragmenting
Schmid	1906	Scrophulariaceae	Micropyle haustoria	Hypertrophied, fragmenting, amoeboid
Huss	1906	Ranunculaceae, Berberidaceae, Papaveraceae	Antipodal cells of embryo sac	Hypertrophied, fragmenting, amoeboid
Guilliermond	1908	Wheat, barley, corn	Cells of embryo	Lobed
Friedsohn	1910	Frog	Polymorphic, leucocytes, etc.	Lobed, fragmenting
Němec	1910	Corydalis cava, Secale cereale, Colutea arborescens	Endosperm	Abnormally large, amoeboid, fragmenting
		Euphorbiaceae	Latex tubes	
Wright	1910	Cat, dog, mouse, rabbit, guinea pig, man	Megakaryocytes of bone marrow	Huge, lobulate
Szily	1911	Animal	Pigmented eye coat, Choroid sarcoma	Irregular, lobed
Maximov	1913	Dog, rat, man, guinea pig	Mastleucocytes	Polymorphic
Samuelson	1913	Empetrum nigrum	Endosperm haustoria	Hypertrophied, amitotic, minute
Schürhoff	1915	Ranunculus acris	Giant endosperm cells	Amoeboid, fragmenting
Jordan	1916	Pig embryo, animal bone, leopard frog	Yolk sac, various types of blood cells	Giant, lobed, amitotic, fragmenting
	1917			
	1918			
	1919			
	1925			
Jenkinson	1916	Lemur	Foetal connective tissue cells	Amoeboid, lobed
Nakahara	1917	Insect larvae	Silk glands	Hypertrophied
Schürhoff	1918	Insects	Adipose tissue	Angular, amitotic
	1917	Asparagus	Cells, periphery of veins	Degenerating, giant, fusing?
Saguchi	1920	Animal	Pancreas, Islets of Langerhans	Irregular, amitotic, canaled?
Bast	1921	Animal	Bone cells	Lobed, amitotic
Champy	1921	Animal	Amebocytes, spermatocytes, epithelium, muscle, excretory cells	Indented, canaled, amoeboid
Sokoloff	1922	Man	Cancer	Irregular, lobed
Vejdovsky	1924	Bee	Adipose tissue	Ramifying
			Oenocytes	Canaled?
Levine	1925	Man	Carcinoma	Hypertrophied, lobulate
Radtke	1926	Euphorbia	Nectaries	Lobed, irregular
Halkert	1927	Ranunculus, tulip, snowdrop	Bulbs, tubers	Angular, lobed
Kater	1927	Snap bean	Cotyledon	Irregular

TABLE II

RELATION OF PARASITIC ENVIRONMENT TO NUCLEAR MORPHOLOGY

AUTHOR	DATE	ANIMAL OR PLANT HOST	PARASITE	ORGAN OR TISSUE AFFECTED	NUCLEAR FORM
Nawaschin..	1899	Cabbage	Plasmodiophora	Roots	Hypertrophied
Toumez.....	1900	Plants	Myxomycetes	Root excrescences	Abnormal,enlarged, fragmenting
Magnus.....	1900	Neottia nidus avis, Orchis	Mycorhiza	Roots	Hypertrophied, amoeboid, canaled?
Tischler.....	1901	Circaea	Nematode	Root galls	Lobed, constricting, fragmenting
Shibata.....	1902	Podocarpus	Root fungi	Roots	Amoeboid, canaled?, fragmenting?
Guttenberg..	1905	Adoxa, Capsella, Zea, Alnus	Puccinia, Ustilago, Albugo, Exoascus, Synchytrium	Fungus galls	Lobed, constricted, branching, canaled
Guttenberg..	1909	Anemone, Mercurialis	Synchytrium	Galls	Canaled
Kusano.....	1909	Pueraria, Amphicarpaea	Synchytrium	Stem and leaf tubercles	Hypertrophied, deformed
Wolpert....	1909	Alnus	Mycorhiza	Roots	Twisted, amoeboid
Arzberger...	1910	Ceanothus, Elaeagnus, Myrica	Mycorhiza	Root tubercles	Hypertrophied, amoeboid
Blomfield...	1910	Veronica	Sorosphaera	Stem tumors	Enormous
Meyer.....	1910	Thismia, Burmannia	Mycorhiza	Roots	Hypertrophied
Němec.....	1910	Coleus, Clerodendron, Pulsatilla, Washingtonia	Nematodes	Galls	Amitotic
Bally.....	1911	Taraxacum	Synchytrium	Leaves	Lobed, canaled
		potato	Chrysophlyctis	Tubers	Lobed, canaled
Osborn.....	1911	Potato	Spongospora	Tubers	Lobed, indented, canaled?
Reynolds....	1912	Viola	Puccinia	Leaves	Deformed, fragmenting
Schürhoff...	1912	Podocarpus	Root fungus	Infected cells	Amoeboid
Spratt.....	1912	Podocarpineae	Pseudomonas	Root nodules	Amoeboid amitotic
Tobler.....	1913	Anemone	Synchytrium	Root cells	Canaled, lobed, fragmenting
Edson.....	1915	Beta vulgaris	Phoma betae	Seedlings	Dumb-bell, budding, amitotic
Kunkel.....	1915	Potato	Spongospora	Tuber	Lobed, distorted
Spratt.....	1919	Leguminosae	Root bacillus	Root nodules	Amoeboid, amitotic
Allen.....	1926	Club wheat	Puccinia	Infected cells	Lobed, dumb-bell, amoeboid

I. Nuclear form

The studies of nuclear form in lilies and other plants have led me to adopt the following terms in describing the various types of nuclear form:

1. NORMAL.—The normal nucleus is ordinarily rounded or oval, with a smooth nuclear membrane inclosing the chromatin granules which lie in the linin network (fig. 1*a*).

2. RIDGED OR IRREGULAR.—The form of the nucleus may be influenced by mechanical pressure of cell inclusions upon it. The nuclear membrane may become irregular in outline because of the

pressure of starch grains upon it, as in the starch storage cells of lily bulb scales (fig. 1*b*).

3. CLEFT.—Lily nuclei very often show a single sharp indentation or deep furrow. Such cleft nuclei are very common in the parenchyma cells of lily leaves, petioles, and stem (fig. 2*a*).

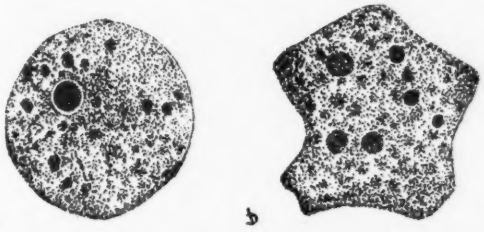

FIG. 1.—*a*, normal nucleus; *b*, ridged or irregular nucleus in storage cell of lily bulblet scale.

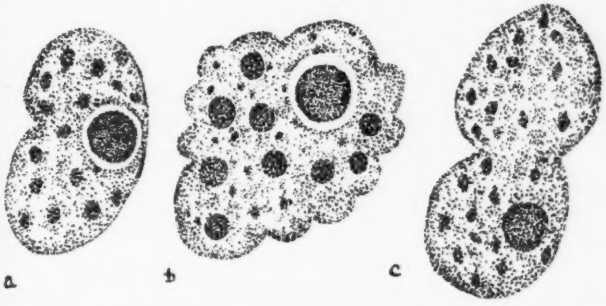

FIG. 2.—*a*, cleft nucleus in lily leaf; *b*, crenate lily nucleus; *c*, constricted lily nucleus.

4. CRENATE.—The crenate form of nucleus is the result of sharp and shallow reticulated furrowing, appearing over the entire nucleus, giving it a nodular surface like that of an orange, but with the elevations larger and more irregular. Such nuclei have been observed in the parenchyma cells of healthy green lily leaves, and in the ground meristem or parenchyma tissue of the flower stems of growing hyacinth bulbs (fig. 2*b*).

5. CONSTRICTED.—The constricted nucleus appears more or less as if it were dividing. Such nuclei occur in the parenchyma cells of

healthy green lily leaves and petioles, and are not an indication of amitosis (fig. 2*c*).

6. SPINOUS.—The spinous nucleus shows sharp-pointed extensions of the membrane over its entire surface. These nuclei usually occur in regions where material is being transferred from tissues in which starch is being digested to tissues in which cell division and differentiation are going on. They occur in lily bulblets, in cells at the base of scales about the point of origin of young roots, and in lily stems below the growing point and leaf primordia. In long hyacinth roots they are found commonly in the ground tissue. In other

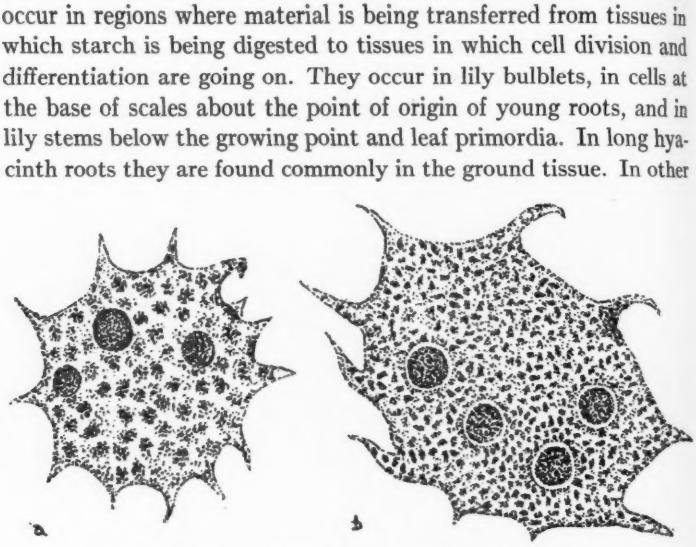

FIG. 3.—*a*, spinous lily nucleus; *b*, spinous hyacinth nucleus

parts of the hyacinth plant they are found in parenchyma cells, by the side of the vascular bundles. KORSCHELT (26) figures a toothed nucleus in a salivary gland of *Chironomus*, in which he observed in living cells that the toothlike processes were put out and withdrawn during the secretory activity of the cells. He believes that there is a gradual passing over through these points of the karyoplasm into the cytoplasm (fig. 3*a*, *b*).

7. BULLATE.—Bullate nuclei show extensions of the nuclear mass in the form of rather coarse blunt projections over their entire surface. They are found in the roots of young lily bulblets, in the parenchyma cells of the mesocotyl of the oat seedling, in the parenchyma cells of the leaves on young sprouting narcissus bulbs, and in the starch storage cells of hyacinth bulbs (fig. 4*a*).

8. BIFURCATE.—The bifurcate nucleus is an elongated one which is deeply indented at each end. These nuclei are found most commonly in elongated conducting cells bordering xylem vessels and tracheids in lily bulb scales, bulblet scales, stems, etc., in narcissus bulb scales, and hyacinth bulb stems (fig. 4*b*).

9. HORNED.—The horned nucleus shows extensions like those of the bullate nuclei, but larger and more irregular. They are found in lily bulblets, and in the growing points below leaf and branch primordia. In lily and hyacinth roots, large round meristem cells above the growing points contain nuclei which, instead of bearing

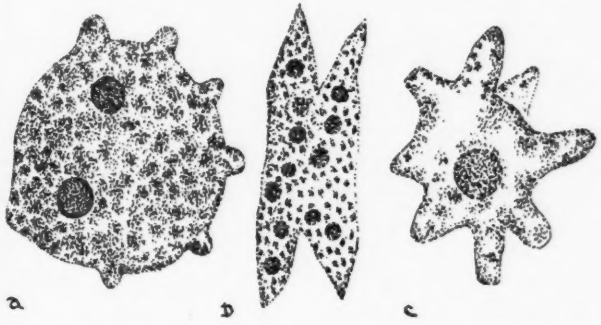

FIG. 4.—*a*, bullate lily nucleus; *b*, bifurcate lily nucleus; *c*, horned nucleus in epidermal cell of buckwheat endosperm.

fine teeth, may show tapering coarser projections or horns (fig. 4*c*). In the old bulb scales of hyacinths, after nearly all the storage starch has been removed, the nuclei appear collapsed, hypertrophied, and show one or several such hornlike projections.

10. FURROWED OR CANALED.—So-called canaled nuclei are not really perforated by tubular canals running directly through the nuclear substance. The canals referred to by various writers are simply furrows (seen in section) through the nuclear mass formed by the folding in of the nuclear membrane. This form of nucleus is undoubtedly associated with secretory activity. Such nuclei were found in the cells of bulb scales of the lily and hyacinth when starch was being digested, in the cells of the cotyledons of germinating

peas, soy beans, and bush beans, in the carpellary cells of the iceberg blackberry, in the parenchyma cells of sprouting dahlia tubers in

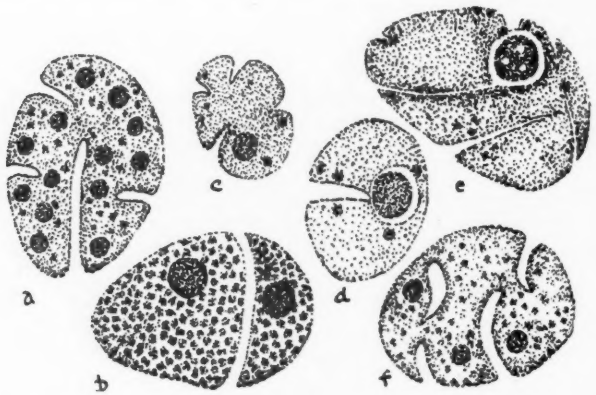

FIG. 5.—*a*, furrowed lily nucleus; *b*, furrowed nucleus in dahlia tuber; *c*, furrowed nucleus in endosperm of buckwheat; *d*, furrowed nucleus in corn endosperm; *e*, furrowed nucleus in bush bean cotyledon; *f*, furrowed nucleus in hyacinth root.

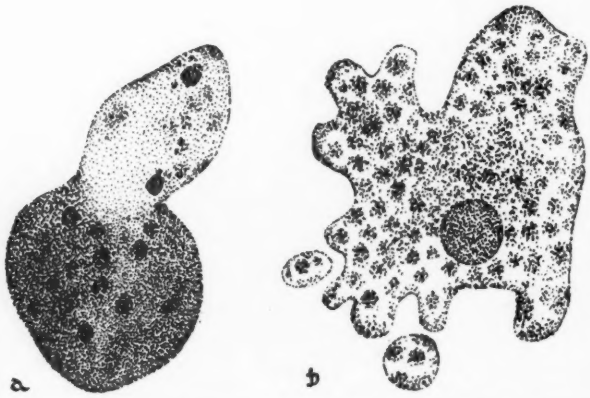

FIG. 6.—*a*, amoeboid nucleus in yellowing lily leaf; *b*, lobulate nucleus in starch parenchyma cell of yellowed lily leaf.

the endosperm cells of sprouting corn and buckwheat, and in the mesocotyl cells of the oat seedling (fig. 5*a–f*).

11. AMOEBOID.—The amoeboid nucleus suggests by its form that there has been some thrusting out of pseudopod-like extensions associated with a flowing movement of the karyoplasm. It has usually been applied to a lobed nucleus which resembles an amoeba with pseudopodial extensions. KORSCHELT's observations (**26**) perhaps warrant such an assumption. It may very well be that all the various forms of nuclei if observed in living condition would be found to be changing their forms in a fashion more or less amoeba-like (fig. 6*a*).

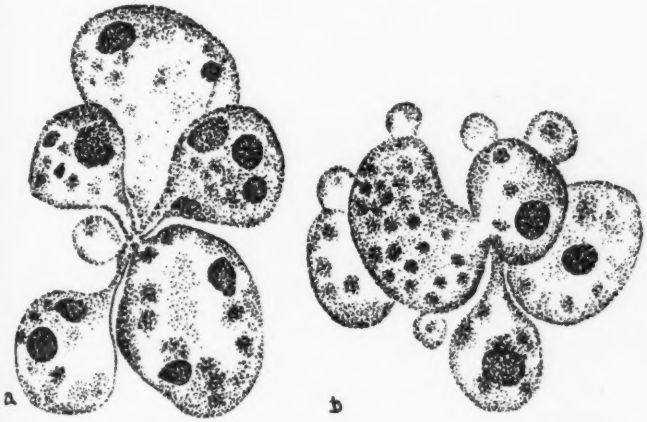

FIG. 7.—Lobed nuclei in starch parenchyma cells of yellowing lily leaves

12. LOBULATE.—The lobulate nucleus shows wide, rounded extensions over more or less of its surface. Fig. 8*a* shows a nucleus of a blackberry carpel that is furrowed, while in fig. 8*b* the nucleus appears lobulate. In fig. 8*c* the lobulate nucleus in a hyacinth flower stem also indicates that it derived its lobulate form from a widening out of narrower furrows. Lobulate nuclei, like furrowed nuclei, are found in regions of digestion, and may represent a higher stage of digestive activity than do the furrowed nuclei. Lobulate nuclei are found in the starch storage cells of lily bulb scales after growth has started, in the starch sheath about the veins of lily leaves, in hyacinth bulb scales, carpels of the iceberg blackberry, storage

cells of pea cotyledons, and in hypocotyls after growth has started (fig. 6*b*).

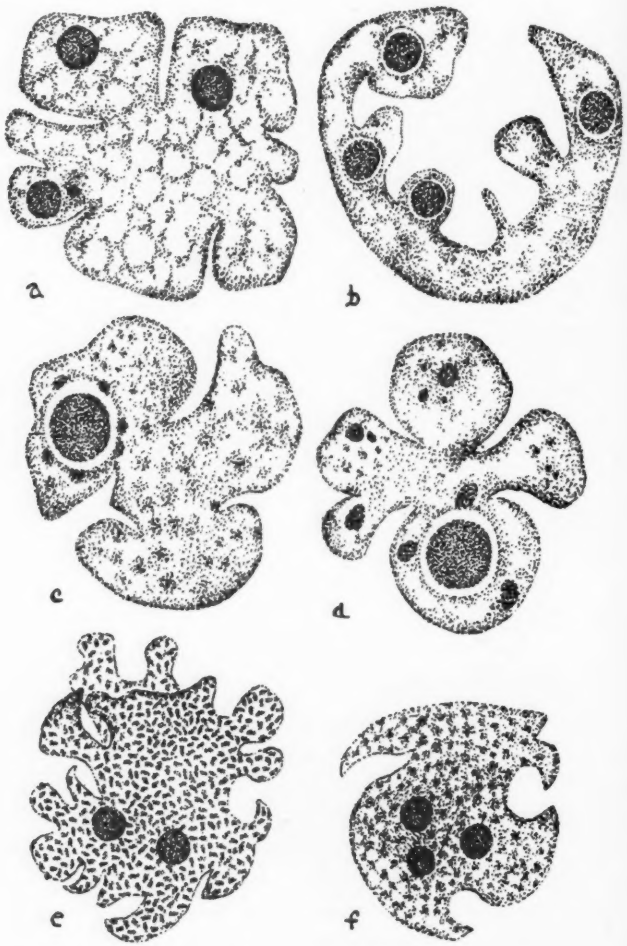

FIG. 8.—*a*, canaled nucleus; *b*, lobulate nucleus (cells of iceberg blackberry carpels, courtesy of B. O. DODGE); *c*, canaled nucleus; *d*, lobed nucleus (cells of pea cotyledons); *e*, lobulate nucleus in hyacinth flower stem; *f*, furrowed (lobed?) nucleus in narcissus leaf.

13. LOBED.—I have distinguished a still more exaggerated type of amoeboid form as typically lobed. In such cases only slender connections unite the lobes, which are thus almost distinct nuclear fragments. This lobed effect may be brought about by a simple widening and deepening of the grooves in a furrowed nucleus. Such a nucleus is almost broken up into a number of separate masses of nuclear material. Lobed nuclei are associated with the same activities as are furrowed and lobulate nuclei, and they occur in the same tissues (fig. 7*a*, *b*).

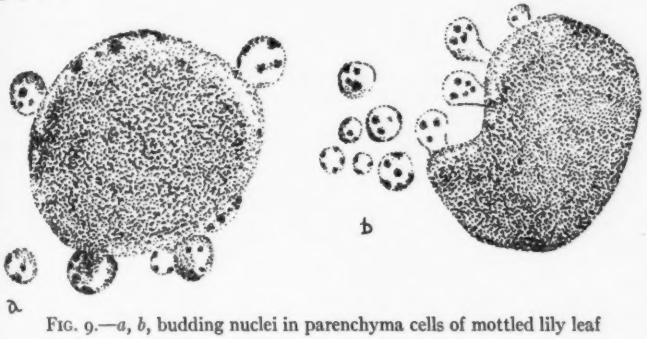

FIG. 9.—*a*, *b*, budding nuclei in parenchyma cells of mottled lily leaf

14. FRAGMENTING, AMITOTIC, AND BUDDING NUCLEI.—Lobing may lead to a final breaking up of the nuclear body into two (or perhaps more or less) rounded fragments. In thin sections the cell may appear to have several or many nuclei, merely because the attachments between the parts of a lobed nucleus are not included in the section (fig. 10*g*). Fig. 10*f* shows a single rounded large nucleus with two smaller rounded nuclei near it. Figs. 9*a* and 9*b* show many minute nuclei apparently budded off from a large rounded nucleus. Such cases of budding nuclei were only observed in lily material.

All these types of nuclei are to be regarded as normal, and specialized in connection with some particular metabolic activity. A later paper will describe certain appearances associated with nuclear degeneration.

II. Nuclear form in cells of diseased lily plants

1. NUCLEAR FORM IN CELLS OF BULBLETS OF DISEASED LILIES.—The bulblets studied were found attached to the mother bulbs of

lily plants that were either stunted in growth, or whose leaves were completely yellowed or blotched, suggesting a virus infection. The bulblets were growing and possessed several young roots. The forms of nuclei found in their various tissues were in general similar to

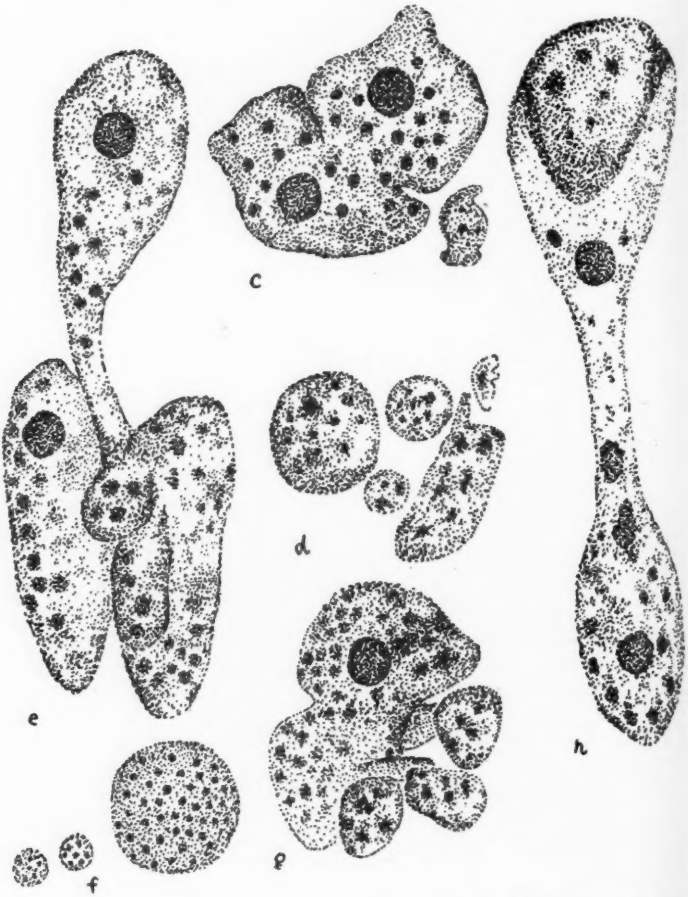

FIG. 10.—*c*, constricted nucleus and nuclear fragment in cell of lily leaf; *d*, group of irregular lily nuclei after fragmentation of hypertrophied nucleus; *e*, lobulate nucleus in xylem parenchyma cell of lily stem; *f*, large rounded nucleus and two smaller nuclei appearing together in lily leaf cell; *g*, lobed nucleus appearing fragmented because of sectioning; *h*, elongated nucleus in xylem parenchyma cell of diseased lily stem.

those described for the cells of healthy bulblets. In elongating cells, destined to become vascular elements, the nuclei appeared to be degenerating, and these cells also contained various kinds of degeneration or secretion deposits. I shall give elsewhere the results of a cytological study of the histogenesis of these vascular elements, with reference to the origin and development of various inclusions (slime bodies, spiral and spindle-shaped structures, wax plates, etc.) which occur in such tissues.

2. NUCLEI IN APPARENTLY HEALTHY LILY PLANTS.—Material was studied from large, apparently healthy bulbs of *Lilium longiflorum*, *L. giganteum*, and *L. harrisii*, which were purchased from commercial seed companies. Certain parts of such bulbs were fixed and sectioned immediately after removal from storage, other parts later on after growth had begun. This material shows clearly that irregularity in nuclear form is associated with increased activity in the plant. In the case of the resting bulbs, the nuclei in the thick outer scales, thinner inner scales, bulb stems, and growing points were found to be rounded in form, and normal in appearance and size. Moderately toothed forms occur in the pith and cortical parenchyma cells of the stem, which contain small grains of storage starch. In the elongated conducting cells spindle-shaped and bifurcate nuclei are found.

In bulbs that have started growth, lobed nuclei are found in the storage parenchyma of the outermost scales, where starch digestion is taking place. In elongated conducting cells below the growing points, the nuclei appear fragmenting by a pinching off of small rounded nuclei. Many cleft nuclei are found in the parenchymatous cells of the young leaves. In the young roots the nuclei are normal excepting near the growing point of the root, where they show minute toothlike projections.

The nuclei of the palisade and parenchyma cells of the leaves are generally normal in appearance, and may be slightly indented or cleft. Some of the nuclei may even be lobed. In the cells of senescent leaves from the base of otherwise healthy lily plants, the nuclei may be lobed and irregular in the spongy parenchyma cells. Many cells appear binucleate. Some of these cells contain grayish deposits or secretion bodies of an apparently oily nature. These bodies can be distinguished from the x-bodies of tobacco mosaic by the irregularity of their outlines.

3. NUCLEAR FORMS IN CELLS OF MOSAICED LEAVES.—Many of the lily plants show unquestionable symptoms of mosaic, with typical *x*-bodies in the leaf cells (fig. 11). The plants have not been studied from the standpoint of their possibly contain-

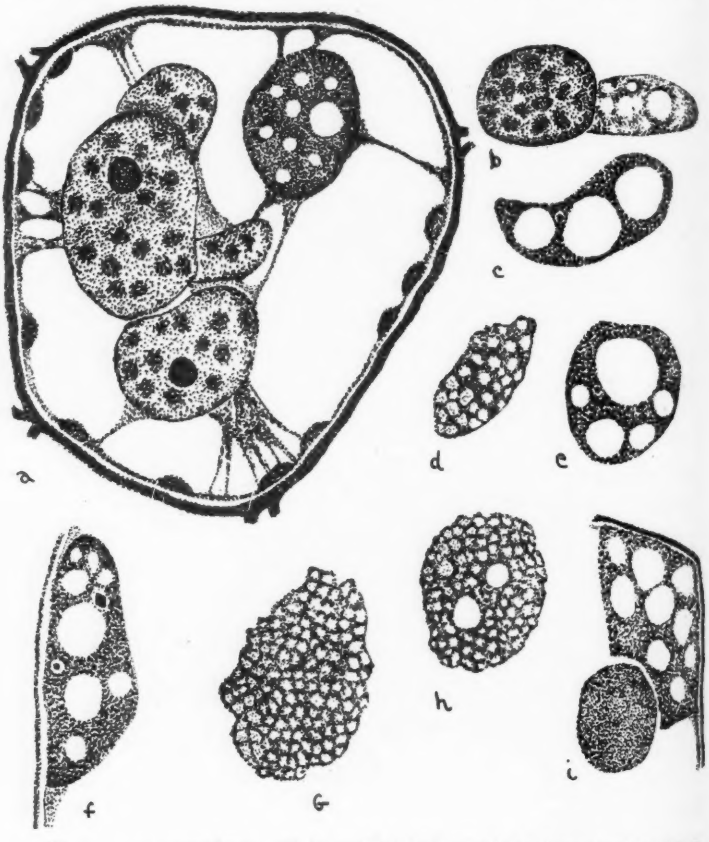

FIG. 11.—*a*, parenchyma cell in mottled leaf containing hypertrophied lobed nucleus and large vacuolate *x*-body; *b*, vacuolate *x*-body attached to normal nucleus; *c*, vacuolate *x*-body; *d*, secretion body; *e*, *x*-body including two red staining granules; *f*, large *x*-body containing two cuboidal red stained protein grains and red stained rounded granule; *g*, *h*, secretion bodies; *i*, vacuolate *x*-body in corner of cell, with dead shrunken red-stained nucleus pressing against it (by focusing, the *x*-body can be seen quite distinct from primordial utricle).

ing a transmissible virus, or as to the general course of the disease. The starch in these diseased leaves is in general found only in parenchyma cells about the vascular bundles. The nuclei in these cells, where digestion of the storage starch is going on, are very irregular in form, and appear hypertrophied, amoeboid, or deeply lobed. These cells often contain small nuclei, which may have been formed by the budding of larger nuclei, or by fragmentation of lobed nuclei. In some of the parenchyma cells there are red staining bodies lying in the cytoplasm that appear to be extrusions of the chromatin or nucleolar material from the nucleus. Many nuclei are markedly irregular in outline, suggesting flowing movements of the karyoplasm. In these leaves the secretion bodies (fig. 11*d*, *g*, *h*) found in senescent leaves also appear. The *x*-bodies are unusually large, take the orange stain, and contain several large rounded vacuoles (fig. 11*a*).

In connection with the studies on the cytology of plants affected with virus diseases (10, 11, 12), I have noted the general absence of irregular or hypertrophied nuclear forms. This is in strong contrast with the general situation as to plants infected with intracellular parasites. This leads one to believe that the irregular and hypertrophied nuclei in mottled and yellowing lily leaves are not due to the presence of the virus but to the dying condition of the leaves.

4. NUCLEI IN DISEASED GROWING POINTS AND STEMS.—In the growing points of plants which appear diseased and stunted in growth, exceedingly hypertrophied nuclei are found in the ground meristem and parenchyma cells about and between the vascular bundles. In these cells also occur grains of storage starch, which show evidence of digestion in their irregular outline, and possibly products of digestion in the form of orange and red staining granules. Huge twisted nuclei or lobed forms occur in the xylem parenchyma cells, and the elongated conducting cells contain bifurcate nuclei. Large *x*-bodies occur commonly in the parenchyma cells about the veins. Grayish, apparently oily secretion deposits are also present in these cells.

Conclusion

These cytological studies of plants infected with virus diseases impress one with the fact that the nucleus is hardly affected structurally by the diseased condition. At the most it has been found in

tobacco that the disease is associated with a slight increase in size, and a very slight irregularity in outline of the nucleus. Tobacco and dahlia mosaic tend rather to be inhibitive and destructive to the cell elements as well as to the plants. Many intracellular parasites, such as the chytrids, induce hypertrophies and hyperplasias. The virus diseases tend rather to stunt the development of the cell and of the entire organism, even killing the cells in some cases, although in general mosaiced plants are able to reach maturity and set seed.

The very numerous and striking cases of nuclear deformation in specialized tissues in the lily are at least not obviously associated in any way with the presence of the x-bodies. The nucleus is the center of all physiological activity of the cell, and becomes during periods of very active and special phases of metabolism exceedingly hypertrophied and irregular in form. Such hypertrophied nuclei with cleft, crenate, or furrowed membranes, or lobed, lobulate, or amoeboid outlines, occur normally in healthy plants, in special organs of nutrition, and associated with certain phases of nutritional and secretory activity.

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PLANT SUCCESSION AND ECOLOGICAL HISTORY OF A CENTRAL INDIANA SWAMP

STANLEY A. CAIN

(WITH EIGHT FIGURES)

Bacon's Swamp, with an approximate area of 30 acres, lies in the E 1/2, SE 1/4 of Section 6, Washington Township, Marion County, Indiana. A rather narrow arm of the swamp runs north and south. The southern end of the swamp swings southwest and broadens out to somewhat more than a quarter of a mile in width. This retort-shaped basin is obviously of glacial origin, as it is entirely inclosed by slopes exposing glacial material. In general the upland is level or slightly undulating. There are a few places in the vicinity with moderate morainal topography. Glacial boulders occur at the surface in relatively few places. Bacon's Swamp is a unique feature of the vicinity, which is without lakes or swamp except for two very small swampy areas, one a mile away and the other half a mile distant. There are, of course, swampy places along the floodplains of the streams. The basin in which Bacon's Swamp lies is a constructional feature of the Bloomington Ice Sheet of Early Wisconsin glaciation. MALOTT (7) shows a morainal region in the middle eastern part of the county. Bacon's Swamp should probably be assigned to this feature.

According to GEIB and SCHROEDER (6), Marion County includes three distinct geological formations: Carboniferous limestone in the eastern part of the county, which is underlain by black Genesee shale in the west, and Knob sandstone in the southwest. These rock formations, however, are covered to a depth of 50–100 feet by a deposit of drift which forms the surface of the county and determines the character of the soils. The soils of Marion County belong to the Miami and Huntington series. The Miami soils have been derived directly from the glacial material which covers the whole region. The Huntington soils are alluvial. Bacon's Swamp lies in the Miami clay loam, which is by far the most extensive soil, oc-

cupying 76 per cent of the county. The Miami black clay loam occupies only a small percentage of the county, and occurs in small basin-like depressions throughout the upland.

The material composing the Miami black clay loam is of glacial origin, but since its deposition it has been modified to a considerable extent. Prior to the construction of drainage systems sufficient to carry off the surplus water, these small areas were covered with swamps and marshes. Through the growth and decay of vegetation large amounts of organic matter have been added to the soil, the rapid oxidation of which was prevented by excessive moisture. Some of the finer particles of soil have been washed into these depressions from the surrounding upland, and this has had considerable influence on the texture of the soil.

Only two areas of muck are mapped in the survey of Marion County, and these are very limited. The material consists of vegetable matter in advanced stages of decomposition, with fine earth from the higher surrounding land intermixed. The muck is black, and extends to a depth of from 18 inches to 4 or 5 feet, usually resting upon a bed of clay. There is no mention in the soil survey of any peat deposits in the county. Evidently the presence of Bacon's Swamp was unknown to these men, since, as has been stated, the swamp is a unique feature of the area.

The basin in which the present swamp lies is lined with a fine compact blue silt which has been washed in from the surrounding soil. Borings around the edge of the swamp have reached this practically impenetrable layer at a depth in places of only a few inches. Sometime ago Mr. DOUGLASS (5), of Trevlac, Indiana, made borings through the peat in the center of the swamp. In recent correspondence with the writer he stated:

> I prospected Bacon's Swamp for the peat and invented a machine for compressing it for fuel purposes, back about 1905. My boring outfit had only 35 feet of pipe and I failed to touch bottom at the deepest place, all solid peat. The entire swamp is underlaid with a heavy, water-retaining, blue clay. I have no idea how thick this layer may be but it serves to hold the water in the swamp as perfectly as though it were a crockery bowl.

The final recession of the Bloomington ice sheet (perhaps 60,000 years ago) no doubt left a lake at the present site of Bacon's Swamp.

From the time the last ice left this latitude, and the small post-glacial lake was formed, the vegetational history has been in progress. As stated by BLATCHLEY (1):

> A lake of small size begins to die the moment it is born. Its basin begins to fill with material other than water, and the process of final extinction is commenced. There are more beds of extinct lakes in northern Indiana today than there are existing ones. These former basins are now sites of bogs or meadows underlain by 15 to 29 or more feet of muck and marl.

Bacon's Lake is now extinct. The margin is grown up with water-inhabiting trees and the interior is occupied by a wet meadow. The vegetation of Bacon's Swamp presents some intriguing aspects. An understanding of the relationships of the various plant associations depends upon a knowledge of the past vegetational history of the area. It would be better, perhaps, to describe the vegetation as it now exists and then proceed to certain evidences of the earlier vegetational conditions.

Present vegetation

The center of the swamp is occupied by a wet meadow ordinarily inundated from 8 to 15 inches, and dominated by *Calamagrostis canadensis*[1] with an abundance of *Dulichium arundinaceum*, *Juncus canadensis*, *Aspidium thelypteris*, and *Hypericum virginicum*. There are two places where this meadow is interrupted. The first is a rectangular area of open water near the center of the swamp where it bends to the southwest (figs. 1, 2). This pond is the result of an attempt to construct a road across the swamp. In the fall of 1914 the township built a dirt and gravel road, now known as 56th Street, from College Avenue on the west to Keystone Avenue on the east. The following winter the portion of the road bed constructed on the peat across the wet meadow of the swamp disappeared, the weight of the road bed compressing the loose water-soaked peat.

The vegetation about this open water would be quite puzzling if the origin of the pond were not known. The plants of the wet meadow grow almost to the edge of the open water, from which there is a very abrupt transition. The normal series, from submerged hydrophytes through rooted hydrophytes with floating leaves to

[1] The nomenclature is that of GRAY'S *New Manual of Botany*, 7th ed.

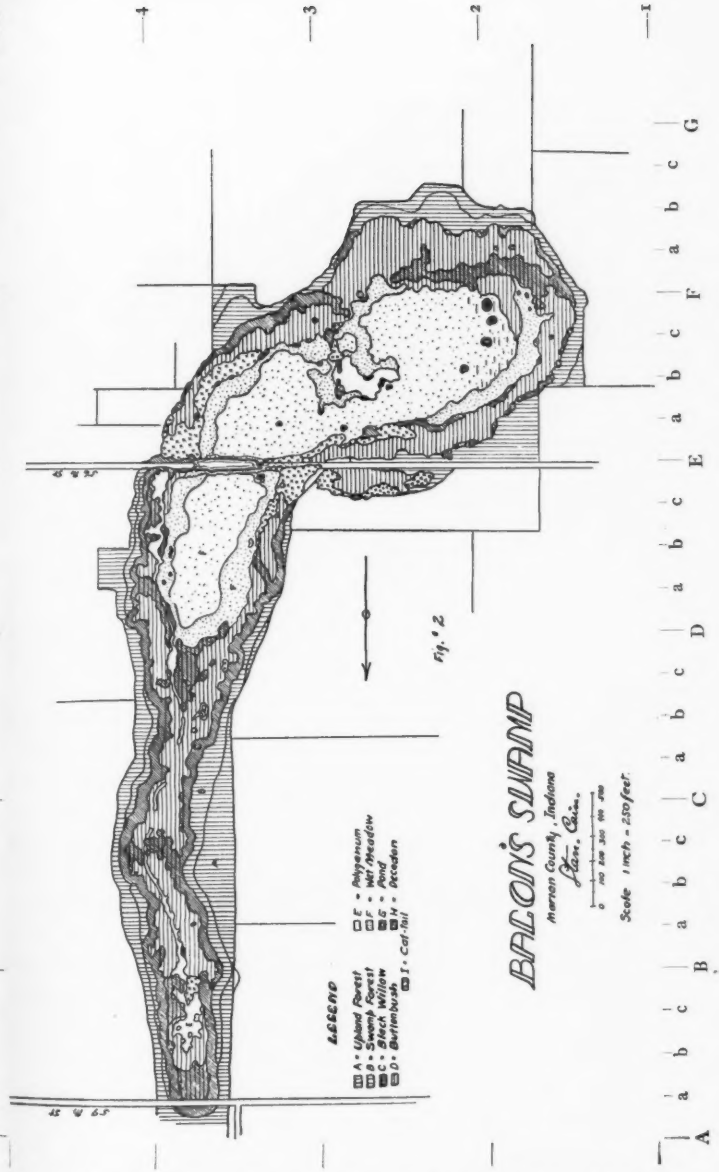


FIG. 1.—Vegetation map of Bacon's Swamp derived from airplane photographs (2): letters and figures at sides of map facilitate location of photographs and references in connection with this study; for dimensions apply the reduced scale of map itself.

erect hydrophytes, as *Typha*, etc., is not represented here. The absence of these stages is explained by the abruptness with which the substratum drops off from water about a foot or less in depth, where the typical meadow is found, to a depth too great for *Nymphaea*, etc., within a horizontal distance of 3 or 4 feet. However, a few scattered plants of *Cephalanthus* are making their appearance

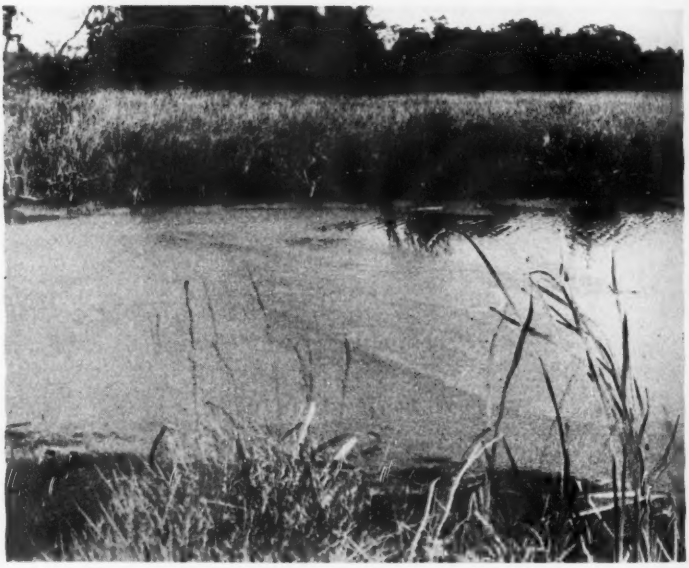


FIG. 2.—(Ea-3.5, N)* The 56th Street pond, showing abruptness with which *Calamagrostis* meadow stops, eliminating all stages from meadow to open water, except for scattered specimens of *Cephalanthus* which are just appearing.

* The parenthetical numbers and letters in this and following figure legends indicate exact location and direction of photograph in map (fig. 1); for example, N (north) indicates direction in which camera was pointed.

along the north side (fig. 2). In addition to the buttonbush, a few scattered species, as *Polygonum hydropiperoides* and *Scirpus cyperinus*, are to be found. As the area of the open water is not great there is practically no wave action, and also there is little inwash of material, except at the ends of the pond, as the peat is fibrous and bound by roots; thus the abruptness of the sides of the depression is very slowly changing.

The other region with open water is found in about the center of the wide southwest end of the meadow, and is connected with the moat on the south by a growth of *Typha*, *Polygonum*, *Carex*, etc., occupying water too deep for the plants of the ordinary meadow. This region is of quite a different type from the one just described. In the first place it is entirely natural and unaffected by man. It lies over what is probably the deepest part of the basin (over 35 feet deep), and is the last to be filled by vegetational deposition. The substratum is very light and spongy, so much so that it cannot be traversed. The first plants to appear around the open water are *Polygonum hydropiperoides* and *P. muhlenbergii*, rooted in the muck. *Nymphaea advena*, *Sagittaria latifolia*, *Ranunculus delphinifolius*, etc., do not appear here as they would under similar conditions elsewhere. The explanation for this is probably revealed by studies of the hydrogen-ion concentration (CAIN 3). This region is about 100 times as acid as is the region previously discussed, or as is the usual habitat of these plants.

Near this hole occur clumps of shrubs, as *Salix discolor* and its variety *eriocephala*, *S. sericea*, and *Cephalanthus occidentalis*. It is a significant feature of this region that scattered patches of *Decodon verticillatus* occur along the edge meadow-mat next to the open water (fig. 3). This fact, together with the presence of *Sphagnum* remnants in the peat, indicate typical bog conditions sometime in the past.

Scattered over the swamp in a number of places are hydrophytic associations which are typical for drained swamps. These regions, which are to be found mostly between the meadow and the buttonbush, have the ordinary freshwater succession. Usually the first stage of submerged aquatics is poorly represented, probably because of the peaty nature of the substratum. For example, *Ceratophyllum*, *Myriophyllum*, *Vallisneria*, etc., seem to be entirely absent. This stage is here represented practically entirely by algae and diatoms, and various microhydrophytes.

The second stage, composed of rooted hydrophytes with floating leaves, is characterized by *Nymphaea advena*. Another plant which should be placed here is *Ranunculus delphinifolius*. A later development in the succession is that of the erect hydrophytes, rooted in the

muck and raising their leaves above the water. The species of *Polygonum* seem to appear first in this stage. There are three species occurring at Bacon's Swamp, *Polygonum hydropiperoides*, *P. muhlenbergii*, and *P. sagittatum*. *Sagittaria latifolia* is a very common member of this group. *Typha latifolia* seems to occur later, and tends to replace the previous plants. The explanation of this

FIG. 3.—(Eb-2.7, NW) An "island" of *Decodon* near edge of open water in southwest lobe of swamp.

immediate succession probably lies in the shading of the broader leaves of the lower-growing plants by those of the cat-tail, which rise to considerable height. Thus the broader-leaved and lower-growing plants suffer, in their turn, the fate they meted out to the plants with floating leaves which preceded them. The other important factor is that of the depth of water, each stage filling in with its own detritus until the depth will permit the growth of the succeeding stage. The matter of shading, however, within limits of depth, seems to be the controlling factor. *Typha* is found growing

in water from 1 to perhaps 2 feet deep. At Bacon's Swamp *Typha* seems to occupy a rather unusual position, between the hydrophytes just described on the one hand and the wet meadow on the other, while in places it is intermediate between the wet meadow and the buttonbush. Of the two, the buttonbush enjoys a wider distribution than the cat-tail; growing in deeper water, it forms a practically continuous zone about the wet meadow inside the willow zone (fig. 1).

There is a very interesting group of small floating plants deserving special mention, including *Lemna minor, L. trisulca, Riccia fluitans*, and *Wolffia columbiana*. The different members of this group can be found almost anywhere in the swamp (that is, in the meadow, the buttonbush zone, or the ponds) varying in relative abundance from one season to the other. Fig. 2 shows the surface of the 56th Street pond practically covered with *Wolffia*.

The wet meadow, which occupies the whole center of the swamp, with the exceptions previously noted, grows on the highest part of the peat. For the last 2 years the center of the meadow has been covered with 8 inches or more of water. During previous drier seasons the water has been reduced to below the surface of the peat for part of the year. During the drier periods fires have sometimes occurred, producing considerable holes in the surface layers of the peat; however, submergence seems to be the usual condition of the meadow. Toward the outer edge the water becomes deeper. At about 12–15 inches the dominant *Calamagrostis canadensis* and its associates give way to *Carex impressa*. The sedge flourishes to a depth where a pure stand of *Cephalanthus occidentalis* is to be found. This stocky shrub grows in water 2–4 feet or more in depth. It is most characteristic for these plants to show an abrupt transition, but in some places they intermingle or *Typha* is intermediate.

From the interior of the swamp one can see quite distinctly the concentric zones of vegetation, each rising somewhat higher than the one next inside it. Standing in the *Calamagrostis* meadow one sees first the *Carex* zone, then successively the buttonbush, black willow, and the swamp and upland forests in the rear. Some of these zones are indicated in figs. 4 and 5.

Former vegetation

The southwest end of the swamp is the most interesting portion. It is here that the concentric zones of vegetation are broadest, and their relations to each other and their probable historical development are clearest. One unique feature of this region is seen in the "islands" of *Decodon verticillatus* (figs. 3, 6). These islands are rem-

FIG. 4.—(Eb-2, N) View from interior, showing some of concentric zones indicated at margin: (a) *Calamagrostis* meadow, (b) *Cephalanthus* zone, (c) *Salix nigra* zone, (d) swamp forest.

nants of former conditions when Bacon's Swamp was occupied by a typical *Sphagnum* bog association. In the fall of 1927, and again in 1928, a little *Sphagnum* was able to reestablish itself after a dry period, but there had been none for a few years before, and this was not able to maintain itself. The surface peat shows, on microscopic examination, a considerable admixture of *Sphagnum* along with remnants of the typical meadow plants of the present; but the underlying peat can be described only as Mr. DOUGLASS has done, as

"a pure *Sphagnum* peat." At present the *Calamagrostis* meadow comes to the margin of these islands but seems unable to eliminate them, although the water is usually less deep. This is undoubtedly due to their habit of rooting at the tips of the branches, which results in a dense tangle of plants. These descending branch tips show fine development of aerenchyma where they are submerged. The surface

FIG. 5.—(F-2.4, W) Another interior view of swamp, showing extension of black willow into central part; zones indicated thus: (a) *Salix*, (b) *Cephalanthus*, (c) *Carex impressa*.

water in this vicinity is considerably more acid than it is at the other end of the swamp. This is due to the still greater acidity of the underlying peat, which reaches a pH of 4.4. The southwest end of the meadow differs from the rest in another respect. Quantities of *Cephalanthus* are scattered throughout, particularly between the *Decodon* and the *Carex* (fig. 1). A close examination of these clumps of buttonbush shows that these plants have at no distant time experienced better living conditions. Everywhere the shoots rise from

among dead branches of a more luxuriant growth (fig. 7). In all clumps examined the root systems were heavier than the present growth would warrant, and old stems were present. There seems to be only one explanation: for some reason the water level has recently been changed so that *Cephalanthus* is losing out, and the meadow is coming to occupy a greater portion of the area. The same phenomenon probably also accounts for the rapid disappearance of

FIG. 6.—(Ea to Fa and 1 to 2.5) Airplane photograph of southwest end of swamp, showing "islands" of *Decodon* conspicuous near center (compare with fig. 1; photographed from altitude of 2000 feet).

Sphagnum from the mat. I have studied the conditions of the swamp intensively during the past 4 years, and have not found any living *Sphagnum* except that mentioned. It is very interesting, however, that *Sphagnum* has grown there in abundance until quite recently. Mr. DOUGLASS, who knows the swamp very well, has responded to my inquiries concerning the presence of *Sphagnum* as follows:

It is difficult for me to believe that you now find no *Sphagnum* at Bacon's Swamp. . . . . Certainly it grew there as abundantly and richly as I ever saw

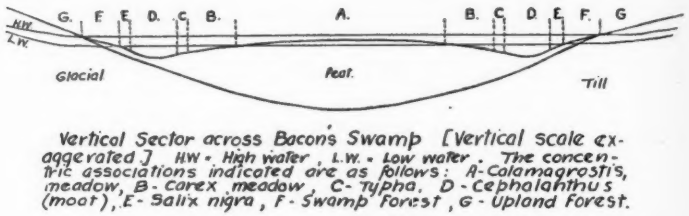
it in a Wisconsin or Michigan swamp, even better. In proof of this you will find that the peat of Bacon's Swamp is a true *Sphagnum* peat, one of the purest *Sphagnum* peats I ever examined, and at one time when I was interested in the subject I examined many samples. Enclosed please find a paper prepared for the Indiana Academy of Science many years ago (DOUGLASS, 1905). All the pictures in this paper were taken at Bacon's Swamp. . . . . The one showing a block of peat is sufficiently clear to show typical *Sphagnum* moss on the top.

FIG. 7.—(Ec-2) *Cephalanthus* from wet meadow; note dead branches characteristic of buttonbush in this region.

Other inquiries led to a similar statement by Professor C. F. Cox, of the Technical High School, Indianapolis, who said, "I have collected *Sphagnum* at Bacon's Swamp as recently as 1919 and 1920." The fact of the rapid disappearance of *Sphagnum*, together with the more gradual restriction of *Cephalanthus* in the southwestern end of the swamp, leads to the conclusion that the water table, in the immediate vicinity at least, has been lowered in the past decade or two. As the region has been practically deforested and under agriculture for almost a century, these factors cannot be used to

explain the recent changes. They are probably due to tilling. The lowering of the water table in the vicinity would affect the drainage of the surface peat in the central meadow. This would hasten the disappearance of *Sphagnum* and the restriction of *Cephalanthus*, and at the same time favor the spread of the *Calamagrostis* meadow.

From a consideration of the depth of the water in the different associations, it is already apparent that, for a swamp, the substratum has an unusual topography (fig. 8). The different depths of the water are intimately related to the plant associations, and become a limiting factor, at least in some cases. The convex surface of the peat, with the greatest height in the center, together with the moat at the periphery, is a further suggestion of the dual nature of the



Vertical Sector across Bacon's Swamp [Vertical scale exaggerated] H.W. = High water, L.W. = Low water. The concentric associations indicated are as follows: A-Calamagrostis meadow, B-Carex meadow, C-Typha, D-Cephalanthus (moat), E-Salix nigra, F-Swamp Forest, G-Upland Forest.

FIG. 8.—Relation of the various swamp associations to water level, high and low; vertical scale greatly exaggerated.

area. It is quite common for even the most typical bog formations to be surrounded by a zone of fresh water plants. Many times in small lakes the bog mat appears, not over the shallower littoral portions of the water, but out from the shore where the water is deeper. So it is that an old lake when practically filled with vegetation, may have a greater accumulation of peat in the center (where it is built under bog conditions of high acidity and poor drainage) than at the periphery, where oxidation and drainage are greater, and the different types of vegetation build more slowly. This would seem to be another evidence of the former acid bog vegetation at Bacon's Swamp. It is likely that the regions now occupied by *Cephalanthus*, *Sclix*, and the swamp forest have been under swamp conditions since the first. The earlier stages of the swamp succession, of which the woody plants represent the culmination, have long since been

eliminated by the presence of the bog mat in the main body of the water.

TRANSEAU (8) notes the fact that the succession from open water to the higher bog margin can be traced easily, but that throughout the region of northern Indiana and Ohio and southern Michigan there seems to be no connection between this succession and the plants of the bordering forests. He states:

> The zonal succession of the plant groups, from the submerged aquatics of the pond to the arborescent forms of the higher bog margin, is clearly defined and well known. But then comes a sudden break, and without a suggestion of gradation the surrounding forest appears.

Since Bacon's Swamp is out of the present range of distribution for most of the common bog trees and shrubs, they would not be expected there within recent times; yet there is little doubt that boreal forms persisted at the swamp long after the main wave of the northern migration had passed by, as they are now found in bogs farther north. Also, this need not interfere with the situation being somewhat as TRANSEAU has described for northern Indiana, for there seems to be even less connection between the meadow and the buttonbush-swamp forest-upland forest succession around the periphery of the depression. This region has probably been under swamp conditions for a very long time, whereas the central part, now occupied by the *Calamagrostis* meadow, has probably been under bog conditions until recently. This fact would account for the apparent hiatus between the succession to the meadow and the succession from the moat to the upland beech-maple climax forest.

COWLES (4) speaks of the marginal flora which is to be found about most types:

> All of the peat bogs have a characteristic marginal flora, i.e., the vegetation at the margin of the original lake is essentially alike in all cases. These plants, as well as those of the *Cassandra* bogs, are the same over wide areas. The most common species of the bog margin flora are *Nyssa sylvatica*, *Populus tremuloides*, *Ilex verticillata*, *Pyrus arbutifolia*, *Spiraea salicifolia*, *S. tomentosa*, *Rubus hispidus*, *Gaultheria procumbens*, *Osmunda cinnamomea*, *O. claytoniana*, *O. regalis*, *Betula papyrifera*, and *Polytrichum commune*. This vegetation originates outside of the swamp, and may be regarded as xerophytic; however, it often encroaches upon the swamp as the latter develops.

It should be said that more than half of the plants just mentioned are found in the flora of Bacon's Swamp, and in general the description fits fairly well with the common marginal flora and development found throughout the region.

Bacon's Swamp is considerably farther south than the present limits of bog distribution (TRANSEAU 8). That Bacon's Swamp, like a number of similar areas outside the present distribution of bogs, has passed a part of its earlier existence and vegetational history under bog conditions is to be expected in connection with the northward trend of vegetation following glaciation. The present study is unique in that the transition from bog to swamp has been so recent, and has been under a degree of scientific observation during the time. The depth of the basin compared with its surface area has no doubt had some influence on the long duration of the acid bog conditions, so that an unusually deep deposit of peat is to be found there.

Peripheral vegetation

Occupying what I have called the moat is a zone of *Cephalanthus occidentalis*, ranging up to 200 feet in thickness. For the most part this species exists alone, but in places it contains an admixture of *Ilex verticillata*. The *Ilex* nearly always occurs next to the swamp forest and not on the side next to the wet meadow. There is one particularly interesting shrub area on the west side of the swamp (fig. 1, E-2.5 to 2.8). Some buttonbush is to be found there, but it does not characterize the vegetation. Among the other woody plants are *Xanthoxylum americanum*, *Ilex verticillata*, *Aronia floribunda*, *Salix discolor*, *S. discolor* var. *eriocephala*, *S. sericea*, *Rosa palustris*, *Cornus amomum*, *Rubus hispidus;* and a few trees, principally *Acer rubrum* and *Populus deltoides*. With these woody plants may occur quantities of *Aspidium thelypteris*, *Polygonum sagittatum*, *Scirpus cyperinus*, and *Typha latifolia*. In regions where the shrubs are absent and there is not a great amount of shade a typical fen is found, with numerous species, among which are *Lobelia cardinalis*, *L. siphilitica*, *Asclepias incarnata*, *Eupatorium perfoliatum*, *Lycopus uniflorus*, *Apios tuberosa*, *Penthorum sedoides*, *Bidens trichosperma*, and *Saururus cernuus*. There is no other place in the swamp where

there is such an association of plants. An examination of the substratum shows a considerable admixture of sandy soil washed in from the adjacent upland. This region is also subject to considerable fluctuation in the water level, and ranges around the neutral point in acidity.

The swamp forest, which everywhere surrounds the swamp outside the black willow zone, is the last of the zones to be directly connected with the swamp (figs. 1, 8). If *Salix nigra* is considered as a part of the swamp forest, it must be clear that this single species forms a continuous, although narrow zone between the buttonbush and the rest of the forest. In places the willow may even penetrate the buttonbush zone for considerable distances, as along the southern side of the western end of the swamp (figs. 1, 5). The buttonbush does not grow well under the willow, probably because of the shading. In so far as the depth of the water is concerned, neither seems to have an advantage over the other. It would seem from the peripheral location of the willow that it requires a greater admixture of soil with the peat.

The swamp forest proper is made up of *Acer rubrum*, *Ulmus fulva*, *Nyssa sylvatica*, *Fraxinus profunda*, *F. nigra*, *Quercus bicolor*, *Q. palustris*, and *Populus deltoides*. The red maple is to be found throughout the whole region, as is also the elm. Along the north end of the swamp are some pure stands of *Fraxinus profunda*. *Q. bicolor* and *Nyssa* are not found in any quantity. The ordinary woodland herbs are missing from this association, due to the long periods of inundation each season. Along the upper margin of the swamp forest occur *Onoclea sensibilis*, *Carex crinita*, *Ranunculus recurvatus*, *Saururus cernuus*, and *Impatiens biflora*. The upper limits of the swamp forest are the upper limits of inundation (fig. 8). The swamp forest gives way to the upland beech-maple forest which is the climax for the region. Although there is no virgin timber, there are trees large enough to give a good idea of the luxuriance to which this forest once attained.

My lists for the fragments of woodland surrounding the swamp include most of the characteristic species of the upland climax. There are 23 species of trees, including the beech, white and black sugar maple, tulip, poplar, wild black cherry, five species of ash,

four of oak, two of hickory, the Kentucky coffee tree, honey locust, and others. There are 11 species of low trees, forming a second layer in the woods, as follows: two dogwoods, papaw, redbud, blue beech, ironwood, choke cherry, Canadian plum, smooth sumach, and nannyberry. There are seven lianas, including two greenbriers, moonseed, Virginia creeper, poison ivy, trumpet creeper, and summer grape. Besides there are nine species of low shrubs. This gives a total of 50 woody plants in about one-fourth as many acres.

Summary

1. Bacon's Swamp is now occupied by a group of associations characteristic of half-drained swamp areas of the central west.

2. The vegetation in the past has pertained more to the character of undrained swamps. Evidence of this rests on the following points: (a) the luxuriant growth of *Sphagnum* some 20 odd years ago, and its known presence up to 7 years ago;[2] (b) the presence of quantities of well preserved *Sphagnum* remnants in the surface and deeper layers of the peat, particularly in the deeper southwest lobe of the swamp; (c) the higher acidity of the surface water, which is a result of the even higher acidity of the underlying peat; (d) the topography of the peat, which is more characteristic of the type of filling-in found in bogs than in ordinary lake deposition, in that the higher portion of the peat is in the center of the swamp and diminishes toward the periphery, until there is a moat filled with 4 or 5 feet of water which entirely surrounds the wet meadow.

3. There has been a recent lowering of the water table (sometime within the last quarter of a century) which may be taken to account for: (a) the disappearance of the *Sphagnum;* (b) the restriction of the buttonbush to the moat, that is, the elimination of it from the wet meadow, accompanied by the spread of the *Calamagrostis* group.

4. The climatic conditions in the vicinity are such as not to preclude the presence of bog vegetation.

5. The hydrophytic succession, found particularly in the middle and northern, less acid, parts of the swamp, progresses from (a) submerged aquatics; (b) rooted aquatics with floating leaves; (c)

[2] There has been some germination the past two years but no reestablishment.

floating aquatics; (d) rooted aquatics, first with broad erect leaves and later with taller narrow leaves; to (e) the meadow with *Carex* around the deeper margin, and *Calamagrostis*, etc., in the center. These steps are related consecutively to decreasing water depth, with shading as a factor.

6. From *Typha* there seems to be another sequence not definitely related to the one just described, but which starts with the moat and works outward. It consists of (a) *Typha*, (b) *Cephalanthus*, (c) *Salix*, (d) swamp forest, and (e) upland climax forest. The early stages of this sequence seem to have been eliminated by the encroachment of the vegetation of the peat mat in the center. The region of these outer zones has probably never shared in any of the bog history of the central part of the swamp.

It is a pleasure to acknowledge the interest and assistance of Professor HENRY C. COWLES, of the University of Chicago, and Professor RAY C. FRIESNER, of Butler University, Indianapolis.

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NUCLEOLUS IN ROOT TIP MITOSIS IN ZEA MAYS

CONWAY ZIRKLE[1]

(WITH PLATES XII, XIII)

Introduction

In spite of the fact that the nucleolus is in many ways the most conspicuous object in the cell, and has been the subject of numerous investigations during the last 150 years, our knowledge of its composition, function, and behavior during mitosis is most fragmentary. There is little agreement among cytologists who have studied it concerning any phase of its life history. Among the chief points of dispute have been whether or not it was composed of chromatin, and whether or not it contributed to the karyokinetic figure or fragmented and passed out into the cytoplasm. If the nucleolus were in reality two different cell organs, so alike morphologically as to be frequently confounded with each other, there would be a ready explanation for the present confusion. WILSON (16) has indeed provisionally classified the nucleoli into two groups: (1) plasmosomes, or true nucleoli; and (2) karyosomes, or chromatin nucleoli.

Aside from the further complication of the issue by certain workers mistaking clumps of badly fixed chromatin for nucleoli, as WALKER and TOZER (15) have pointed out, undoubted nucleoli have been reported to be composed of chromatin, while others have been recorded as containing no chromatin whatever. There is, of course, no reason for assuming the nucleolus to be composed of the same substances throughout the entire animal and plant kingdoms, yet the evidence for the existence of two distinct types would be more convincing if the same nucleolus had not frequently been reported by different investigators as belonging to each category.

The rôle which has been assigned the nucleolus in the economy of the cell has been quite varied. LUDFORD (6), WILSON (16), and SHARP (12) have summarized these divergent views, so no complete or general account of them will be given here. It may not be amiss,

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however, to list a few of them. It has been held that: (1) the nucleolus is an accumulation of waste products of the nucleus, and is periodically fragmented and passed out into the cytoplasm; (2) the nucleolus is the center of the vital activity of the cell and in fragmenting it carries the chromatin out into the cell; (3) the nucleolus carries the excretory and secretory products out from the nucleus to the cytoplasm; (4) the nucleolus originates in the cytoplasm and serves to carry nutrition to the nucleus; (5) the nucleolus conducts the vegetative processes of the cell; (6) the nucleolus controls the somatic functions, metabolism and movement; (7) the nucleolus is a storehouse for the chromatin of the resting nucleus; (8) the nucleolus is a reserve constituent of the linin reticulum; (9) the nucleolus is a storehouse of nucleic acid to be drawn upon as the chromosomes resume their basophilic character; (10) fragments of the nucleolus pass out into the cytoplasm of the egg and give rise to yolk granules; (11) the fragments of the nucleolus become mitochondria; (12) the nucleolar globules are bearers of stimulating or finishing material of the genes; (13) the nucleolus is a reserve product of metabolic activity and is used in the upbuilding of certain parts of the cell; (14) in the pancreas the nucleolar matter elaborates zymogen; and (15) in certain insects the nucleolus contributes to the activity of the silk glands.

The most common method of cytological investigation of the nucleolus has consisted in examinations of sections fixed by the usual methods, in which the nucleolus was distinguished from other cell organs by its reaction to specific stains. Thus its oxyphilic properties were established. It could in certain mitotic phases be distinguished from the chromatin by this method, as it united with acid dyes when the chromatin was colored by basic ones. The nucleolus has also been reported as staining much more lightly than the chromatin (YAMAHA and SINOTÔ **17**). While these two constituents of the nucleus could thus readily be distinguished in the resting stage, it was generally impossible to separate them in dividing cells, for, as mitosis progressed, the staining properties of the chromatin became more oxyphilic (NAYLOR **11**, KUWADA and SUGIMOTO **3**). From the significant observation that the chromatin assumed certain staining characteristics of the nucleolus as the latter disappeared as

a discrete cell organ, it was inferred that the nucleolus contributed certain substances to the dividing chromatin.

There are certain disadvantages inherent in this technique of investigation. The specific staining reactions of both nucleolar material and chromatin were so conditioned by fixation as to be at times entirely reversed (ZIRKLE 19). Then, too, there was the more serious difficulty of separating these two substances, which are so intimately mixed in living cells, in fixed material where both were preserved. It was inevitable that each substance would obscure the other.

The present investigation is also carried out upon fixed material, and is of course subject to the limitations of all such work. The size, form, and chemical reactions of the nucleoli changed so greatly with variations in the fixatives, that it was not possible to ascertain their true structure by the methods here used. As no attempt was made to check the various fixation images with living sections, no conclusions could be reached concerning either the finer structure of the nucleoli or the exact amount and form of the nucleolar material. It was possible, however, by alterations in the fixing fluids, to fix: (1) the nucleolar material, chromatin, and mitochondria; (2) the nucleolar material and chromatin and dissolve the mitochondria; (3) the nuclear material and mitochondria and dissolve the chromatin; (4) the chromatin and dissolve or render incapable of staining the nuclear material and mitochondria; and (5) the nuclear material and dissolve the chromatin and mitochondria. The nucleolar material could thus be separated chemically from either the chromatin or the mitochondria, and its distribution in the cell investigated unobscured by either of the other two substances.

Whether the colorable material of the resting nucleus in the root tip of *Zea* is localized in the nucleolus or the nuclear reticulum, or in both, depends entirely upon the fixative used. As the substances in the two regions have such different chemical reactions to fixation, no useful purpose can be served by labeling them both chromatin, although both contribute material to the chromosomes. In the present paper the term chromatin will be limited to the stainable material of the reticulum, and is thus synonymous with basichromatin. This is the substance which forms the early spireme and determines certain of the staining properties of the chromosomes. The

nucleolus, by definition, contains no chromatin, and its contents will be referred to as nucleolar material or plastin. While there is very good presumptive evidence that the nucleolar matter is composed of two or more substances, as has been frequently maintained, the methods here used do not separate them readily, so they will be treated as forming a single unit.

The bibliography of the nucleolus is so extensive, and has been so well summarized, that not many references to the literature will be included in the present paper, nor will the present observations be correlated with the previous findings. The earlier work has been reviewed by MONTGOMERY (10), and later work by WALKER and TOZER (15) and LUDFORD (6); YAMAHA and SINOTÔ (17) and GUILLIERMOND and MANGENOT (2) have brought the references up to date. While the methods used in the present investigation may produce evidence of the nucleolar behavior not hitherto available, every specific finding has been both affirmed and denied by numerous workers.

The only tissue examined is the root tip of *Zea mays*, and the single stain used is Haidenhain's iron-alum haematoxylin. Definite conclusions concerning the composition or behavior of the nucleolus in general can hardly be drawn from so limited an investigation of its activities. The methods used, however, may be of more general interest.

Fixation of nucleoli

The effects of pH upon the fixation image of the bichromates has been recorded (ZIRKLE 18). When the fixing fluid is on the acid side of the critical range (pH 4.2–5.2) the image is essentially that of chromic acid; that is, the nucleolus, chromatin, spindle fibers, and spongioplasm fixed; nuclear lymph, mitochondria, and hyaloplasm dissolved. On the basic side of this range the nucleolus, nuclear lymph, mitochondria, and hyaloplasm are fixed and the chromatin and spindle fibers are destroyed. The change from one image to the other is as a rule sudden and complete, the two images being mutually exclusive and the exact point of change being determined by the specific cation. With the bichromates of copper (pH 4.6), glucinum (pH 4.6), and cerium (pH 4.8), the two images overlap with the fixation of chromatin, spindle fibers, nucleoli, nuclear lymph, mito-

chondria, and hyaloplasm. It will be noted that the nucleolus is fixed in both images; in the more acid with the chromatin, and in the one on the basic side of the range with the mitochondria. Nucleolar material can thus be separated from both the chromatin and the mitochondria.

In the acid fixation image the resting nucleus is a hollow body with a centrally located, darkly staining spherical nucleolus, and a periphery composed of the chromatin reticulum (fig. 26). The colorless halo surrounding the nucleolus is thus quite evident. In the more basic image the nucleus is a solid body composed of fixed nuclear lymph in intimate contact with a centrally located nucleolus (fig. 27). There is in this image no halo. The halo surrounding the nucleolus has been reported by numerous cytologists as a distinct structure in the living cell and by others as an artifact. Its existence in the root tips of *Zea* fixed with bichromates is determined entirely by the pH at which fixation occurs. BAILEY (1), however, using the same fixative throughout, has found that this halo exists about the nucleoli in the cambium of dicotyledons, but not about the nucleoli in the cambium of the gymnosperms, which suggests important differences between these two groups.

Unfortunately for an investigation of the nucleolar material unobscured by any chromatin in tissue fixed by the more basic bichromates, its form and distribution in dividing cells are greatly altered; in fact, no dividing cells are found in tissue thus fixed. If a dividing nucleus is so fixed that all chromosomes and spindle fibers are dissolved, the nuclear lymph rounded up and fixed as a single large droplet with the nucleolar material collected into one or two globules, the fixation image is that of a resting nucleus. As the mitochondria are also preserved by the fixatives, any nucleolar material in the cytoplasm cannot be identified with certainty.

The more acid bichromates in general fix the nucleolus in the resting nucleus as a single darkly staining sphere whose diameter is about half that of the nucleus. Mercuric bichromate is an exception, in that when it fixes the nucleoli they are stained brown by the fixative, and remain uncolored by the haematoxylin which stains the chromatin blue. It seems very significant that in material thus fixed and stained the later spireme has a light brown core and a dark blue periphery. When the root tips are fixed with copper bichromate

(pH 4.6) and stained with iron-alum haematoxylin, both chromatin and nucleolar material stain blue. If the sections are mordanted in the fixative, however, in place of the iron-alum the chromatin becomes blue but the nucleoli remain brown. Again the later spireme has a brown core (fig. 25). When fixed with ferrous, zirconium, uranyl, or mercurous bichromate the nucleoli lose their color in destaining before the chromatin. The central region of the nucleoli loses all color before the periphery. As a result they often appear to have a hull about them. This hull has frequently been described.

When the nucleoli are fixed with the more basic bichromates they are the darkest colored and most conspicuous objects in the section. They retain their stain longer than any other cell organ. Their shape and size are the same as when they are fixed by the more acid salts, except that when the alkali earths formed the cations of the fixatives the nucleoli were much smaller, and when lithium was the cation they were amoeboid in shape and had pointed pseudopodia (fig. 28).

Root tips fixed with the various acetates do not show the two distinct images which have been described for material fixed with the bichromates (19). However, changes in the pH at which acetate fixation occurs does cause certain very marked changes in the image. This change is primarily in the nucleolus. When the root tips of *Zea* are fixed with acetic acid, or any acetate more acid than pH 4.0, the nucleoli do not retain the haematoxylin stain and appear large, vacuolate, and colorless. Sometimes the vacuoles will remain faintly blue, although not so blue as the chromatin. The chromosomes are stained much darker than the resting chromatin; thus when chromosome counting is desired, it is possible to destain completely every other object in the section. This increase in the staining capacity of the chromatin as cell division progresses indicates that the difference in the staining properties of resting and dividing chromatin, noted particularly by NAYLOR (11) and by KUWADA and SUGIMOTO (3), is not due exclusively to the incorporation of nucleolar material in the latter, for with these fixatives the nucleolar material, at least in the nucleoli, is colorless. On the other hand, when the tissue is fixed with mercuric or copper bichromate, as has been stated, the dividing chromatin acquires certain of the specific staining reactions of the nucleoli.

On the basic side of pH 4.0 the acetates fix the nucleoli as much

smaller, solid, densely staining bodies. Chromatin is also fixed as long as the fixatives are more acid than pH 4.6. In more alkaline solutions the acetates can be divided into two groups, depending upon their fixing properties. Unlike the bichromates, neither group fixes mitochondria or nuclear lymph, no matter how alkaline the solution. When certain heavy metals form the cations, such as copper, mercury, uranium, and lead, chromatin is fixed by solutions more basic than any solution of bichromate which preserves chromatin. With certain other cations, silver, zinc, cadmium, nickel, etc., the root tip becomes a mere jumble with little recognizable except the nucleolus. Copper acetate (pH 5.2) often fixes resting chromatin in the form of globules. Such "chromatin nucleoli" are shown in fig. 1.

The best fixing fluids for nucleoli so far found are mixtures of bichromates and acetates. The acetates seem to penetrate the tissue more rapidly and thus primarily determine the fixation image. The bichromates harden the image and enable it to pass through the processes of dehydration, imbedding, and sectioning relatively unaltered. The more basic acetates alone, with the exception of those formed by certain of the heavier metals, do not harden the tissue sufficiently to preserve it through the subsequent treatment. The mixtures of acetates and bichromates have the great advantage as nucleolar fixatives of dissolving all mitochrondria; thus in the material they fix there is no danger of confusing any fragment of nucleolar material extruded in the cytoplasm with the mitochondria.

An investigation of chromate and acetate fixation brings to light the importance of the cation in the preservation of the nucleolus. Most of the fluids designed to fix chromatin have no cation except hydrogen. These solutions are satisfactory preservatives of chromatin, but are quite erratic in the fixation of nucleoli. When the cation of a chrome-acetic mixture is one of the group of heavy metals previously mentioned, both chromatin and nucleoli are well fixed and mordanted. The connection between these two substances can then easily be seen. Copper-chrome-acetate (pH 4.6) fixes the chromatin excellently, and so hardens the nucleoli that they cannot be cut, but are thrown out of the cell when hit squarely with the microtome knife. With certain other elements forming the cations of

the relatively basic chrome-acetate mixtures, all chromatin is dissolved and only the nucleolar matter is well preserved and mordanted. There is none of the clumping of nucleolar material such as occurs when fixation is effected by the more basic bichromates. Thus in root tips fixed with nickel-chrome-acetate (pH 5.0–5.2), the nucleolar material can be followed through all of the mitotic phases unobscured by either chromatin or mitochondria.

The addition of formaldehyde greatly influences the fixation images of the acetate. A 2 per cent solution of acetic acid (pH 3.6) fixes the nucleoli as large, vacuolate bodies which do not retain the haematoxylin stain. A mixture of 4 per cent formalin and 2 per cent acetic acid (pH 3.6) fixes the nucleoli as a solid, densely staining body (fig. 2). The image given by a 2 per cent solution of sulphuric acid is not thus altered by the addition of formalin, as the nucleolus thus fixed remains unstained (fig. 3). The mixture of sulphuric acid and formalin fixes in some respects like mercuric and copper bichromate, in that the later spireme and the chromosomes show a core as colorless as the nucleolus in resting cells.

The fixing fluids used in the present investigation are as follows:

1. $Cu(C_2H_3O_2)_2$ ..... 5 gm.
   H_2O ..... 100 cc., pH 5.2 (fig. 1).
2. $HC_2H_3O_2$ ..... 2 cc.
   Formaldehyde 40 per cent .. 10 cc.
   H_2O ..... 90 cc., pH 3.6 (fig. 2).
3. H_2SO_4 ..... 2 cc.
   Formaldehyde 40 per cent .. 10 cc.
   H_2O ..... 90 cc., pH < 1.0 (fig. 3).
4. $HC_2H_3O_2$ ..... 2.5 cc.
   CrO_3 ..... 2.5 gm.
   CuO ..... Slight excess
   H_2O ..... 100 cc., pH 4.6 (figs. 4, 6–13).
5. $HC_2H_3O_2$ ..... 2 cc.
   HgO ..... Slight excess
   H_2O ..... 100 cc., pH 4.2 (figs. 5, 14).
6. $U(C_2H_3O_2)_4$ ..... 5 gm.
   H_2O ..... 100 cc., pH 5.0 (fig. 17).

7.	$HC_2H_3O_2$	2.5 cc.
	CrO_3	2.5 cc.
	$Ni(OH)_2$	Slight excess
	H_2O	100 cc., pH 5.0 (figs. 15, 16, 18–24).
8.	CrO_3	2.5 gm.
	UO_2	Slight excess
	H_2O	100 cc., pH < 1.0 (fig. 26).
9.	Cr_2O_3	2.5 gm.
	SrO	Slight excess
	H_2O	100 cc., pH 5.8 (fig. 27).
10.	Cr_2O_3	2.5 gm.
	Li_2CO_3	3.0 gm.
	H_2O	100 cc., pH 5.2 (fig. 28).
11.	CrO_3	2.5 gm.
	CuO	Slight excess
	H_2O	100 cc., pH 4.6 (fig. 25).

Behavior of nucleolar material during cell division

The plastin in the resting nucleus is concentrated in a single globule, the nucleolus. In root tips fixed with the more basic bichromates, the apparently resting nucleus often contains two or three nucleoli; but evidence has already been cited which indicates that these nuclei are the remains of dividing ones whose chromosomes and spindle fibers have been dissolved, and whose nuclear lymph and plastin have been agglutinated. Whether any strands or fibers connect the nucleolus with the chromatin reticulum in the living resting nucleus cannot be stated. Certainly none exists in those fixed with the more acid bichromates, where the nucleolus is surrounded by a clear, colorless halo, or in those fixed with the more basic ones, where the nucleolus is in intimate contact with the fixed nuclear lymph and the chromatin reticulum is dissolved. No connection exists in acetate-fixed nuclei. When lithium bichromate is used as a fixative, distinct strands connect the tips of the "pseudopods" of the amoeba-shaped nucleolus with the nuclear membrane, but this image departs greatly from the form in the living cell.

In the early prophases, however, a distinct connection is estab-

lished between the nucleolus and the spireme (fig. 2). The connection rapidly broadens (figs. 3, 4), and the nucleolus becomes pear-shaped, with its smaller end connected to the spireme (figs. 6, 7). WAGER (14) has shown this connection quite clearly. By far the best fixatives for the study of this stage are those which preserve both chromatin and plastin, those giving the best results being copper-chrome-acetate (figs. 4, 6–13) and mercuric acetate (figs. 5, 14). Often the smaller end of the nucleolus splits, and thus a double connection with the spireme is established (figs. 5, 8, 15). There is every appearance of the nucleolar material flowing into the spireme, as has been reported from other material by LENOIR (4) and MARTENS (8).

There is also chemical evidence of the nucleolar material entering into the spireme. When the cell is fixed with mercuric bichromate the nucleoli are stained brown by the fixative, and are not colored by Haidenhain's haematoxylin. Later spiremes so fixed and stained have a brown core. Likewise, when the cell is fixed with copper bichromate and mordanted with the fixative in place of iron alum, the nucleoli and the core of the spireme are brown, while the chromatin reticulum and the periphery of the spireme are stained blue. When the cell is fixed with a mixture of formalin and sulphuric acid, the chromatin reticulum and the periphery of the spireme and chromosomes are stained blue with haematoxylin; but the nucleoli and core of the spireme and chromosomes remain colorless. This evidence indicates not only that the nucleolar material enters into the spireme, but that it does so in a particular way, flowing into the spireme just as water does into a collapsed rubber tube. Unfortunately for the hypothesis of this distinct special relationship between the nucleolar material and the chromatin, it is directly contradicted by evidence derived from other fixation. When the root tips are fixed with zinc or nickel bichromate, the nucleoli of resting nuclei are the heaviest staining objects in the cells. Chromosomes thus fixed have colorless cores.

As cell division progresses the chromatin acquires the staining properties of the nucleolus. This has been very clearly shown by NAYLOR (11) and by KUWADA and SUGIMOTO (3). The latter workers ascribe this change in the chromatin's staining capacity to the disappearance of the nuclear membrane. It could equally well be

explained by its taking up of nucleolar material. This latter hypothesis is not necessarily the explanation, however, for when the tissue is fixed with any acetate on the acid side of pH 4.0, the nucleolar material, at least in the resting cells, loses the haematoxylin stain before the chromatin, yet the dividing chromatin retains the stain longer than the resting. The crucial evidence for the nucleolar material entering into the spireme is to be found in cells fixed with nickel-chrome-acetate (pH 5.0–5.2). This fixative dissolves all chromatin and mitochondria, and preserves the nucleolar material. The plastin can thus be seen entering into the spireme unobscured by any chromatin (fig. 22).

The nucleolus, then, connected at two places with the spireme, becomes drawn out into a rod (fig. 9). This rod lies regularly at right angles to the equatorial plate. It becomes drawn out (figs. 10, 16, 17) and dumbbell-shaped (figs. 11, 18). As YAMAHA and SINOTÔ (17) have reported, it then constricts in two. The two parts round up and pass to the poles of the spindle (fig. 12), often remaining connected to the equatorial plate by a darkly staining thread (figs. 13, 19). The thread then disappears and the division is complete (fig. 14). Fortunately for chromosome counting, these bits of plastin do not linger at the equatorial plate.

From the preceding discussion it is evident that the nucleolar material enters the daughter cells in two distinct ways. It is carried over in the chromosomes and also passes over in a distinct body, the latter form reaching the poles first. YAMAHA and SINOTÔ state that this globule of plastin at the pole fragments and all of the pieces pass out into the cytoplasm. The expulsion of nucleolar fragments into the cytoplasm has also been reported recently by LENOIR (5). In *Zea* the globules of plastin at the poles of the spindle can be observed to fragment, and the fragments can be observed in the cytoplasm, although whether they all go out of the nucleus or not cannot be stated; the majority certainly seem to. There is no danger of confusing these nucleolar granules in the cytoplasm with the mitochondria when the material is fixed with nickel-chrome-acetate, as this fixative dissolves all of the latter. Some of these granules are shown in fig. 14.

These nucleolar granules pass into the cytoplasm while the

chromosomes are at metaphase. At this stage the nucleolar material is very thoroughly scattered throughout the cell, that is, in the granules and in the several chromosomes. Figs. 23 and 24 show cells whose chromatin and mitochondria have been dissolved. They are shown in cross-section. The masses of darkly staining granules in the center are of nucleolar material in the chromosomes at the equatorial plate. The granules in the cytoplasm are of the same substance.

As the chromosomes separate at anaphase and go to their respective poles they carry their contained nucleolar material with them (upper cell in fig. 19); thus the second lot of plastin arrives at the poles. As the chromosomes unite to form the daughter nuclei, and as the nuclear membranes are formed, the nucleolar material collects into several droplets (figs. 20, 21). These droplets, united by stainable threads, flow together and form the nucleolus of the resting cell.

The plastin thus is continuous from cell generation to cell generation, like the chromosomes, and like them is formed by an increase in the amount of pre-existing material. In every cell division, however, a certain amount of it, perhaps merely an excess amount, fragments and passes out into the cytoplasm in the form of granules which ultimately disappear.

Discussion

The question naturally arises as to what rôle the nucleolar matter plays in the economy of the cell. The numerous answers quoted in the beginning of this paper evince the interest it has held for cytologists. It is perhaps impossible to determine this function by a mere observation of the behavior of the plastin during cell division, and it seems probable that its discovery will have to await the development of new lines of attack. The fortunate circumstances which allowed the chromosomes to be investigated by both the genetic and cytological techniques have increased our knowledge of these bodies out of all proportion to our knowledge of the other nuclear constituents.

Considering how widespread the distribution of nucleoli is throughout the animal and plant kingdoms, they would normally

be expected to perform some universal function. This would not prevent them, of course, from performing in addition certain secondary functions in relatively specialized tissue, such as giving rise to yolk granules in egg cells. We should, however, look for some activity common to all nucleoli to find their primary function. Considering how little is really known at present about the nucleolar material, it would seem that any further speculation concerning its rôle in the cell would be futile, and would be useful only if it served to keep the question open.

During cell division some nucleolar material enters into the chromosomes and is presumably in intimate contact with the genes. In the next division a part of this material passes out into the cytoplasm and disappears. It is tempting to see in this a mechanism for carrying the influence of genes to the organism. There is a specific connection between the nucleoli and the chromosomes, as has been shown by DE MOL (9), who found regularly in *Hyacinthus orientalis* two nucleoli in diploid, three in triploid, and four in tetraploid forms. It must be remembered, however, that there is no evidence as yet that the genes need any such mechanism.

The plastin may serve merely as a framework for the distribution of chromatin to the daughter cells. In resting nuclei the chromatin is electro-negative and the nucleoli are electro-positive (KUWADA and SUGIMOTO 3, WILSON 16). The poles of the spindle form and the electro-negative spireme retreats to the equatorial plane, the maximum distance from the two poles. There it is permeated by the electro-positive nucleolar material until it is mostly electro-positive; whereupon it fragments into chromosomes which split. The chromatin, now bearing a different charge, reverses its previous motion and migrates to the two poles. That the electro-positive nucleolar material is attracted to the poles is shown by the fact that that portion of it which does not enter the spireme is pulled apart into two masses which migrate exactly to the poles (figs. 12–14).

This does not explain why the globules of nucleolar material, once they have reached the poles, should fragment and pass out into the electro-positive cytoplasm; nor does it account for any function they might perform there. It also presupposes that the spireme flattens out on the equatorial plane before it breaks up into chromo-

somes. More definite knowledge concerning this possible function of the nucleolar material should be obtained by further study of the forces involved in cell division.

Summary

By the use of different fixatives it was found possible to fix: (1) chromatin, plastin, and mitochondria; (2) chromatin, plastin, and dissolve all mitochondria; (3) plastin and mitochondria, and dissolve the chromatin; (4) chromatin, and dissolve or render unstainable all plastin and mitochondria; and (5) plastin, and dissolve all chromatin and mitochondria. It was thus possible to investigate the behavior of the nucleolar material during cell division unobscured by any chromatin or mitochondria. In the resting nucleus all chromatin is localized in the reticulum, the nucleolus containing none. After the spireme is formed the nucleolus becomes connected with it, and later becomes pear-shaped, the point of attachment being at the smaller lobe of the pear. As the spireme flattens out on the equatorial plane nucleolar material flows into it. The smaller lobe of the nucleolus is drawn out and splits in two, giving the nucleolus two connections with the spireme. As the nucleolus loses material it becomes rod-shaped, and finally lies at right angles to the plane of division. It is drawn out, constricted in two, and the two fragments round up and migrate to the poles. There they break up into small granules, some of which, possibly all, pass into the cytoplasm where they later disappear. In telophase the nucleolar material which had been incorporated in the chromosomes collects into small globules which later fuse to form the daughter nucleolus. Nucleolar material is thus continuous, and is derived from previously existing nucleolar material. Some, however, is apparently lost during each cell division. Two possible explanations of the function of the nucleolar material are added to those already proposed. (1) The plastin, by entering into the chromosomes, coming into intimate contact with the genes during cell division, and passing in part out into the cytoplasm during the subsequent division, may serve as a vehicle for transmitting the influence of the genes to the organism. (2) The plastin, being electro-positive, changes the electro-negative spireme by flowing into it, to an electro-positive chromatin complex;

thus the chromatin, which had collected at the equatorial plate as far as possible from the poles of the spindle, reverses its motion with its electrical charge and migrates to the two poles.

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EXPLANATION OF PLATES XII, XIII

All figures are taken from longitudinal sections of root tips of *Zea mays* except figs. 22, 23, and 24, which are from cross-sections. Haidenhain's iron-alum haematoxylin is the only stain used. Magnification, ×1200; no photographs retouched.

PLATE XII

FIG. 1.—Fixed with $Cu(H_3C_3O_2)_2$ pH 5.2, showing resting chromatin changed into "chromatin nucleoli."

FIG. 2.—Fixed with mixture of acetic acid and formalin, showing connection between nucleolus and spireme.

FIG. 3.—Fixed with sulphuric acid and formalin, showing connection between pear-shaped lightly staining nucleolus and darkly staining spireme.

FIG. 4.—Fixed with copper-chrome-acetate pH 4.6, showing connection between nucleolus and spireme.

FIG. 5.—Fixed with mercuric acetate pH 4.2, showing split in small end of pear-shaped nucleolus and double connection between it and spireme.

FIGS. 8–10.—Fixed with copper-chrome-acetate, showing rod-shaped nucleolus moving to position at right angles to equatorial plate.

FIGS. 11–13.—Fixed with copper-chrome-acetate, showing division of nucleolar material; globules arrive at poles while chromatin is still at metaphase.

FIG. 14.—Fixed with mercuric acetate, showing chromatin at metaphase and globule of plastin at pole; some fragments of nucleolar material in cytoplasm.

PLATE XIII

FIG. 15.—Fixed with nickel-chrome-acetate pH 5.2, which preserves plastin but dissolves all chromatin; nucleolus shown attached to spireme.

FIG. 16.—Fixed with nickel-chrome-acetate, showing nucleolar material at right angles to spireme.

FIG. 17.—Fixed with uranium acetate pH 5.0, showing nucleolus attached to spireme.

FIG. 18.—Fixed with nickel-chrome-acetate, showing nucleolar material divided but still at right angles to equatorial plate.

FIG. 19.—Fixed with nickel-chrome-acetate, showing (1) two masses of plastin in anaphase in upper cell; and (2) globules of nucleolar material passing to poles prior to division of chromosomes.

FIGS. 20, 21.—Fixed with nickel-chrome-acetate, showing plastin globules in daughter nuclei before fusing to form daughter nucleoli.

FIG. 22.—Cross-section fixed with nickel-chrome-acetate, showing nucleolar material passing out into spireme.

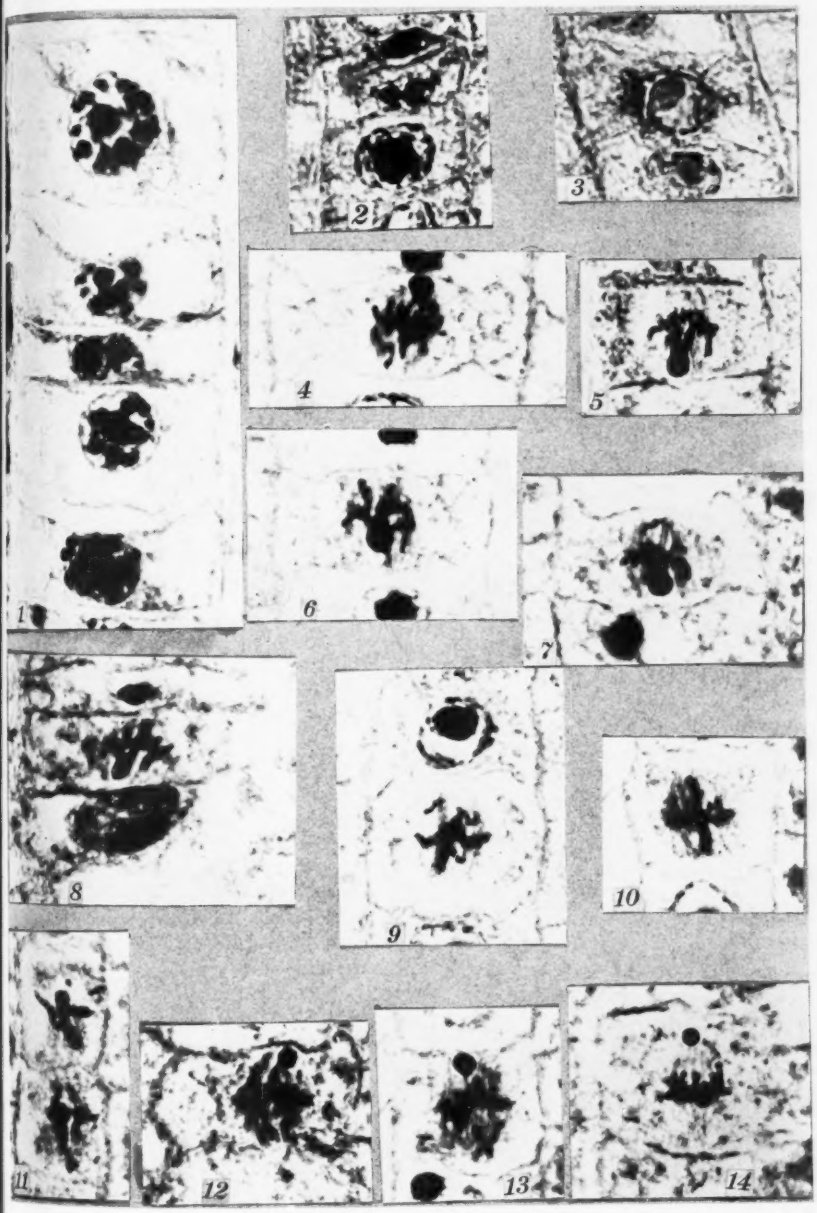
FIGS. 23, 24.—Cross-section fixed with nickel-chrome-acetate, showing polar views of equatorial plate; dark masses in center of cells composed of nucleolar matter left behind by dissolved chromatin; cytoplasmic granules also of nucleolar matter.

FIG. 25.—Fixed with copper bichromate pH 4.6 and mordanted with fixative instead of iron-alum; lighter regions of spireme of nucleolar material.

FIG. 26.—Fixed with uranyl bichromate pH < 1.0, showing halo about nucleoli.

FIG. 27.—Fixed with strontium bichromate pH 5.8, showing absence of halo and chromatin.

FIG. 28.—Fixed with lithium bichromate pH 5.2, showing amoeba-shaped nucleoli.



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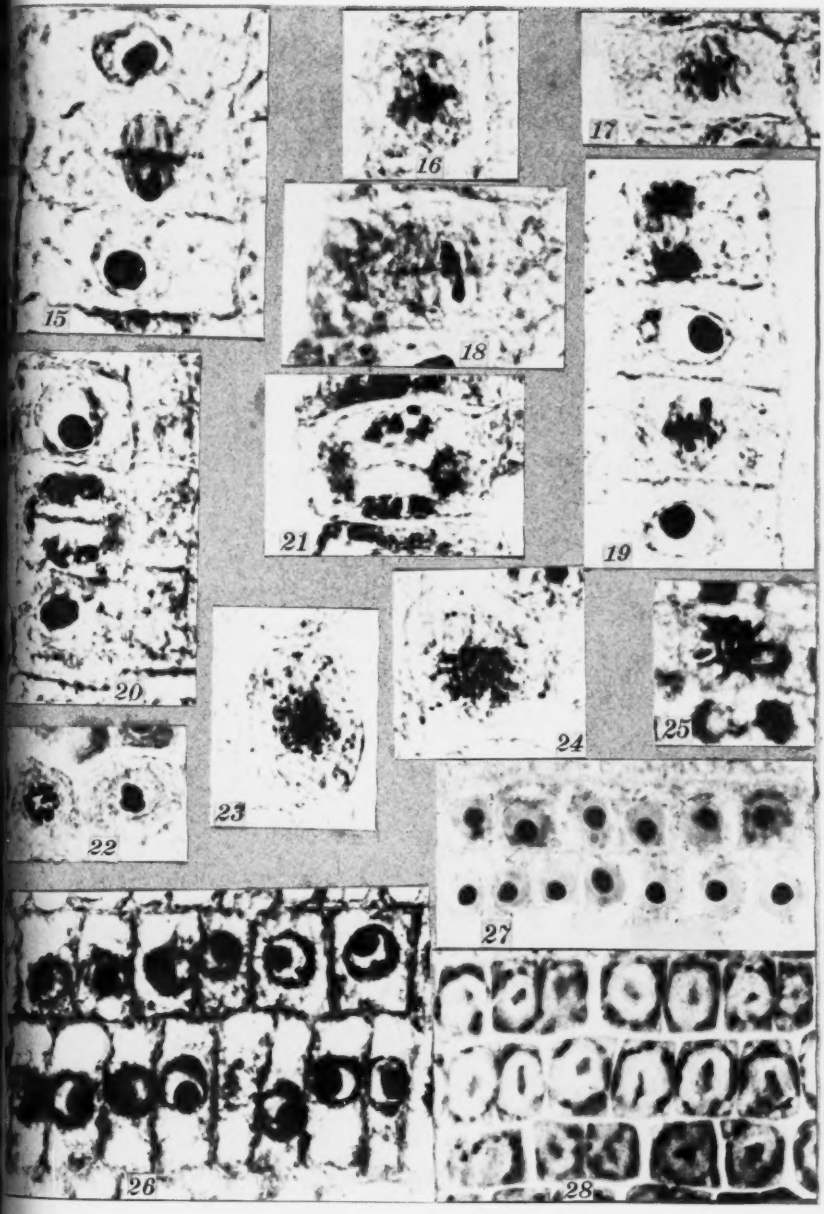

ZIRKLE on ZEA MAYS

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PELVETIA FASTIGIATA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 387

LAURA BROOKS MOORE

(WITH TWENTY-FIVE FIGURES)

Introduction

In a study of a western American species of *Pelvetia*, the writer observed that the conceptacles occurred, not only at the fruiting tips of the shoot, as in *Fucus*, but also much farther down, being rather numerous as far as the second and third forks, and scattered sparsely still farther from the growing tips. These conceptacles were found to contain well developed oogonia. As this condition does not occur in the European species, *Pelvetia canaliculata*, this investigation was undertaken for the purpose of determining the nature of the scattered conceptacles and their relation to hair pits. Other problems arose, resulting in the study of the apical cell, the anatomy of the thallus, and the development of the conceptacle and the sex organs of *Pelvetia*.

The genus *Pelvetia* was founded by DECAISNE and THURET in 1845 (3). J. G. AGARDH assigned to *Pelvetia fastigiata* its specific name in 1848. During the following 25 years no work on *Pelvetia* was published. In 1875, KNY (7) described the apical cell of *P. canaliculata*, and in 1878 THURET (17) published his classical work on the conceptacle of *Fucus serratus* (L.), *F. vesiculosus* L., and *F. platycarpus* Thuret; *Ascophyllum nodosum* (L.) Le Jolis, *Pelvetia canaliculata* (L.) Lyngb., *Bifurcaria tuberculata*, and *Cystoseira fibrosa* (Huds.) Ag. All species of *Fucus* were shown to develop 8 oospheres in each oogonium; *Ascophyllum* was shown to develop 4 eggs; *Pelvetia*, 2; and each of the other genera one oosphere in each oogonium.

OLTMANNS (12) was the first to investigate the Fucaceae adequately from a morphological standpoint. His work was revised in 1904, and further revised in 1922–1923. He confirmed the work of THURET but extended his investigations much further, finding that

8 nuclei are formed in the oogonium of *Pelvetia canaliculata*, and that 2 become centers for oospheres, while 6 are extruded at the equator. OLTMANNS studied the development of the conceptacle carefully in many species of the Fucaceae, but not in *Pelvetia*. He also made thorough investigations of the development of the sporelings of many Fucaceae, including that of *P. canaliculata*, which he found to be like the other Fucaceae in general. There is a 3-sided apical cell which becomes 4-sided with the broadening of the thallus, but from this point the development is unlike that of *Fucus*. *Fucus* stands nearer the common ancestor because its thallus is comparatively simple; its higher number of eggs points to an earlier origin. *Pelevetia* is more highly developed in that the number of eggs has been reduced to two, but in anatomical structure it has not gone so far as *Fucus*. This is another case, according to OLTMANNS (13), where sex organs have progressed and vegetative organs have stood still or retrograded.

The development of the conceptacle in the Fucaceae has also been studied successively by REINKE, who worked on species of *Fucus;* by BARTON (1), who studied *Turbinaria;* and by Miss ROE (14), who investigated the conceptacle in species of *Fucus* and in *Splachnidium*. The most important contribution was made by BOWER (2), however, working on various species of the Fucaceae, including *Pelvetia canaliculata*, work which has been generally accepted. The earlier investigators held with KÜTZING that the conceptacle was derived from the epidermal layer of the thallus, but the others confirm for the most part the views of BOWER, who found that the conceptacle was the product of basal cells cut off from the epidermal cells, together with cortical cells. Miss SIMONS (15) differs radically from all of them in her claim that a single initial of the epidermal layer gives rise to the entire inner wall of the conceptacle from which sex organs spring.

The first cytological work was that of FARMER and WILLIAMS (4). They studied chiefly species of *Fucus* and *Pelvetia canaliculata* in a supplementary way, giving special attention to egg formation in the oogonium, the third division, fertilization, and early segmentation divisions.

STRASBURGER (16) made a cytological study of the third division

in the oogonium of *Fucus platycarpus*, and fertilization in *F. serratus* and *F. vesiculosus*. Most of our knowledge of the cytology of these algae has as its basis the brilliant work of these men.

YAMANOUCHI (18) published a paper on mitosis in *Fucus* based upon *F. vesiculosus*, and sums up the results of his study as follows: The chromosome number is 64, reduced at the end of the second nuclear division in the oogonium and antheridium initials; each of the 4 nuclei at the end of the first 2 divisions contains 2 univalent chromosomes, and this number persists until the formation of the sperm and egg; the phase containing 32 chromosomes may be regarded as the gametophyte generation, and the 64-chromosome phase after the fusion of the gametes as the sporophyte generation.

The American species, *Pelvetia canaliculata*, was studied by HOLTZ (6). He investigated the development of the conceptacle, the apical cell, and the formation of the oospheres. His views on the development of the conceptacle differ from those of BOWER, in that HOLTZ thought that the basal segments of the epidermal cells and also 5 or 6 rows of the cortex beneath them disintegrate, as well as a row of epidermal cells; and that the conceptacle cavity is formed mainly from cortical cells. HOLTZ represented the division of the contents of the oogonium in the same plane as the European species, *Pelvetia canaliculata*. He was able to see only 4 nuclei in each egg.

NIENBURG (10), in an article on the development of the conceptacle in the Fucaceae, criticized HOLTZ' conclusions as to the manner in which the conceptacle of *Pelvetia fastigiata* is developed. NIENBURG secured the American species from California and made a careful study of it. He concluded that the conceptacle arises from a single surface cell which remains behind its neighbors in cell division, so that very early it is sunken. The initial does not have a flask shape, as in other Fucaceae, but is rather even in breadth and ends in a rounded point. Also, in the 2-nucleate stage the nuclei do not lie side by side. The first division is by a longitudinal wall; then follows, first in one and then in the other initial half, a transverse division. From the two basal cells arises through irregular cell divisions the floor of the conceptacle. The upper portion of the initials grows out into short hairs. The side walls begin to be formed through division of the neighbor cells, as in *Fucus*. That other cells

assist in forming the older stages is not certain but probable, according to NIENBURG. He agrees with SIMONS (15) that there is no dying off nor disintegration of cells; but SIMONS thought that the cortex played no rôle in the formation of the conceptacle wall, while NIENBURG claimed that the side walls of the conceptacle originated from the cortex and basal cells of the epidermis. NIENBURG does not mention the peculiar "tongue" cell and "cup-shaped" cell which SIMONS found in *Sargassum*.

KYLIN (8) contributed two interesting articles on the structure of sperms in the Fucaceae. The nucleus divides until 64 are formed, as shown by YAMANOUCHI (18). In the 2- and 4-nucleate, and also in the 64-nucleate stages he observed orange chromatophores, one for each nucleus. In the chromatophores of the Fucaceae, according to KYLIN, there are 3 dyes besides chlorophyll: carotin, xanthophyl, and fucoxanthin, only the first two being found in the eyespot of the sperm. Sperms of *Pelvetia* have no eyespots, however, according to OLTMANNS.

Investigation

The western American species was identified by the writer as *Pelvetia fastigiata* (J. Ag.) de Toni. This genus was founded upon the *Fucus canaliculata* of LINNAEUS (Syst. Nat. II. 1759. p. 716), based largely upon the fact that the oogonium produces but 2 viable eggs instead of 8, the characteristic number for *Fucus*. Eight nuclei are formed within the oogonium, but 6 of them are extruded. The eggs are not fertilized outside of the oogonium as in *Fucus*. The fronds are firm and flattened, becoming terete with age toward the base, and arise from a disk-shaped holdfast; there is no midrib; the branching is dichotomous, diffuse, and fastigiate; the growing point is apical; reproductive organs are developed in conceptacles; oogonia develop two eggs; it is monoecious; color is yellowish brown to dark olive-green.

Pelvetia fastigiata is always found growing on rocks a little below the tide line, where it is sprayed by the surf. It is submerged not more than three hours out of twelve, and OLTMANNS (12) suggests that it might be classed as a land plant for this reason. Its range extends from Coos Bay, Oregon, to the west coast of lower California. It is probably the same species as the *P. wrightii* of Japan.

Plants of *Pelvetia fastigiata* vary in size. At San Pedro, plants 10 dm. long have been observed. On the west coast of the Monterey Peninsula, the type locality, the branches are wide-spreading, angles rounded, and some of the fronds measure 2 cm. wide near the base.

The material for this study was collected by Professor C. J. CHAMBERLAIN near Redondo, California, in September, 1927; and by Dr. A. W. HAUPT at three different locations at different times, namely, San Pedro (October, 1924), Paleo Verdeo (February, 1926), and at Laguna (July, 1926). The material was stained with Haidenhain's iron-alum haematoxylin.

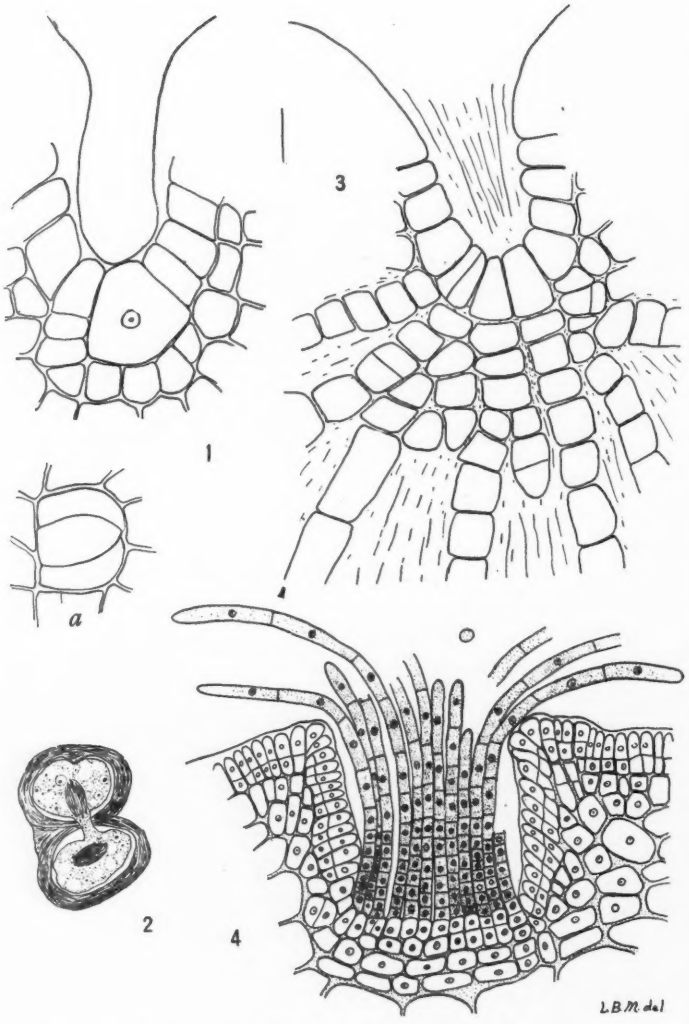
ANATOMY OF THALLUS

Only adult material was used. Attention was given to the apical cell, the general structure of the thallus, hair pits, and conceptacles.

APICAL CELL.—The form of the apical cell is a four-sided, truncated pyramid. The first segment cut off is basal; then follow walls to the right and left which cut off two segments from the opposite sides of the pyramid. Next a wall comes through to one side of the center at right angles to two and three. The segments thus formed are usually wider than the preceding lateral segments, which makes the apical cell longer than wide as seen in longitudinal sections (fig. 1). The segments are not angular but have convex walls, probably due to the greater density of their contents (fig. 1 *a*). The apical cell is sunken in a cleft at the apex, with sides parallel to the flattened surface of the thallus, as in other Fucaceae. This cleft is not so deep in *Pelvetia*, however, and its bottom has a more broadly rounded contour. The cleft and the mucilage which fills it give protection to the tender growing point. The depression is formed by growth as the segments are cut off from the apical cell on all four sides.

THALLUS.—There are three regions of the thallus: the epidermal region, the cortex, and the central cylinder or pith region.

The epidermal layer consists of a single row of palisade cells, much longer than wide, and occurring in pairs, due to their mode of longitudinal division. An epidermal cell first cuts off a nearly cubical basal portion; then the upper portion divides longitudinally, resulting in extremely long slender cells, while the basal cell usually does not divide in this plane, so that we have twin epidermal cells



FIGS. 1–4.—Fig. 1, apical cell in longitudinal section, *a*, in transverse section (×415); fig. 2, cells of cortex (×635); fig. 3, apical region showing pith filaments (×415); fig. 4, hair pit (×415).*

*All drawings reduced one-half except figs. 19–25 which are reduced one-third.

with one basal cell. The basal cell then cuts off segments from its lower surface. The palisade cells are rich in plastids and carry on photosynthesis; also, some plastids occur in the basal cells and even in the cortex. The plastids are oval or ellipsoid, regular in size, and are packed very closely in the epidermal cells. The epidermis is covered by a thick sheath of mucilage.

The cortex has its origin from the basal segments of the epidermal cells. The cells are quite irregular in size but always larger than the basal cells. They are rounded, and the intercellular spaces are filled with mucilage. Their function is storage and conduction (fig. 2).

The central region is a very loose web of filaments of large, elongated pith cells. *Pelvetia* has no midrib, and the central cylinder is composed of mucilage and pith filaments in about equal proportion, the mucilage showing distinct striations in stained preparations. There are infrequent air spaces but no air vesicles, and, as *Pelvetia* is not in water for more than a fourth of the time, air vesicles are not especially needed. The filaments of pith cells are from two to six cells long, sometimes longer. The cells are rectangular, and have very thick but delicate walls formed by successive thin layers or lamellae, like walls of laminated bast fibers (fig. 3). They are not thickened in the way that conductive cells are thickened, but a series of delicate layers seems to have been laid down by the cytoplasm. There is evident protoplasmic connection between the cells of the web through large openings in the cell walls, which are easily seen, and through which the protoplasm passes. This also occurs in the cortical cells. All of the pith cells have nuclei. The function of the pith filaments seems to be conduction, and in lower parts of the stem they have the pointed ends characteristic of conductive cells. The filaments are branched, and form an irregular network traversing the mucilage which fills the central region, some filaments ending blindly in the mucilage. Pith filaments arise from the basal segment of the apical cell.

Hyphae are confined to the holdfast and a space 2 or 3 cm. above it in the stele. They are the strengthening fibers that give mechanical support; and in many Fucaceae that grow where they are swept by violent waves hyphae continue the length of the plant.

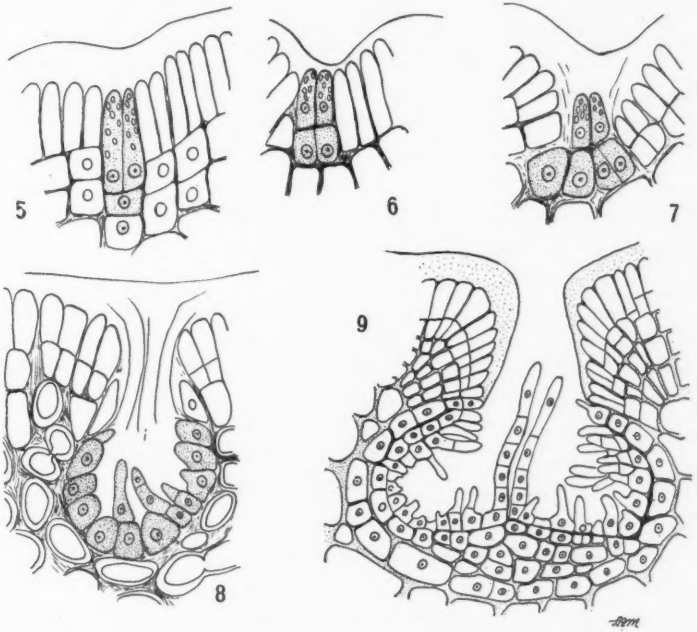
Pelvetia is not subjected to this strain and is not provided with the strengthening tissue. Its vegetative tissue is simple, with little differentiation.

HAIR PITS.—*Pelvetia fastigiata* shows a few indubitable hair pits, although they have never been reported for this genus (fig. 4). They differ from fertile conceptacles in several particulars and may readily be distinguished. They are of different shape. A median cross-section through a conceptacle shows a bowl-like form with a comparatively narrow mouth, without which the conceptacle would be spherical. The hair pit has a broad mouth and straight side walls in transverse section, and the bottom is almost straight across, so that it is rather rectangular than spherical. No conceptacles of this shape were found. Again, the hairs of the hair pit are larger in diameter and are full of darkly staining contents, while the hairs found in conceptacles are smaller and nearly colorless. The longer hairs of the conceptacles of *Pelvetia* grow more commonly on the sides near the mouth of the conceptacle; whereas in the hair pit the hairs have their vegetative bases in the floor of the pit. The hairs of pits project several times their own length, while the hairs of conceptacles rarely extend much, if any, beyond the ostiole. The hairs of hair pits are divided into cells, short at the base, and gradually longer toward the apex. The base broadens into a vegetative basal cell imbedded in the floor of the pit. Their function probably is to increase the absorptive capacity of the plant.

CONCEPTACLES.—Conceptacles are located at the tips of fronds, as in *Fucus*, but the fruiting portions do not present so much of a swollen appearance, and there are also many conceptacles as far down as the second and third dichotomy, and a few scattered ones below.

The initial cell appears depressed, on account of more rapid growth of the neighboring cells, and there arises a slight depression in the mucilage layer surrounding the epidermis. Next, the initial cell divides longitudinally (fig. 5); both daughter cells cut off basal cells; the basal cells continue to divide and form the wall of the bottom of the conceptacle (fig. 6); meanwhile division and growth take place in the neighboring cells. The pair of initial cells is soon at the bottom of a pit twice their length and growing deeper, the

sides of which are formed at first by epidermal cells forced to assume new positions by the great activity of growth taking place in the bottom of the forming conceptacle (fig. 7). These epidermal cells form the walls of the upper part of the conceptacle which eventually



FIGS. 5–9.—Fig. 5, early stage of conceptacle formation; fig. 6, second stage showing division of basal cell; fig. 7, each half of basal cell divided; fig. 8, later stage, shaded cells of floor of conceptacle are derivatives of basal cell; fig. 9, later stage: ostiole lined with epidermal cells, floor of conceptacle and genetic layer are from basal cell, upper cells of the two initials divided to form hairs, sex organ initials and hairs beginning to appear (×415).

is the ostiole, and the basal segments of the initials form the entire inner wall from which later the sex organs come (fig. 8). The shape of the young conceptacle in early stages is similar to the shape of the groove at the apical region when seen in cross-section, which is not surprising because the same sort of activity is present there. In maturity the conceptacle becomes globular except the ostiole or

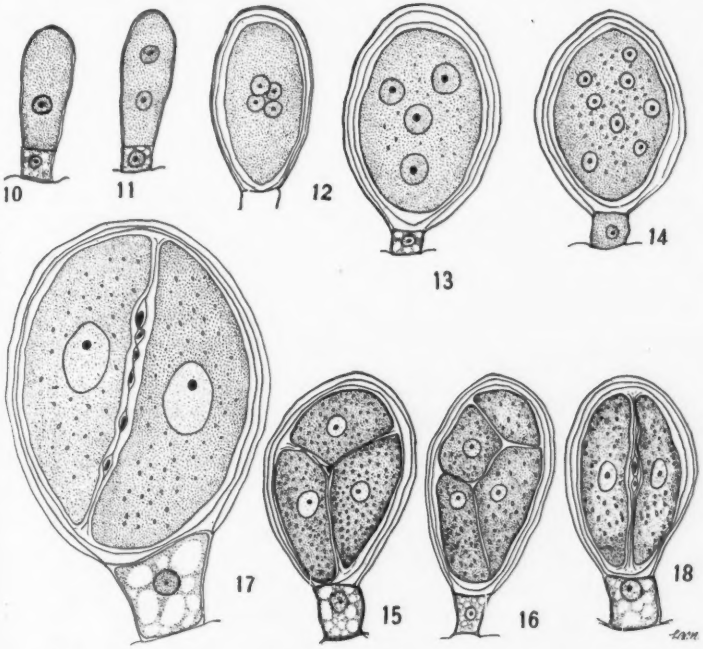
opening, which is narrow. The divisions of the basal segments of the initials form a mound in the bottom, and there are various protruding initials in the early stages (fig. 9); but at the stage of maturity when oogonia and antheridia are ripe the floor is even and its cells are flattened. The upper segments of the initials also divide and grow into two filaments several cells in length, which function apparently as hairs in the conceptacle (fig. 9). They pass through the life cycle of normal hairs, gradually losing their contents, then their transverse walls, and finally becoming colorless sheaths. This is the history of other hairs of the conceptacle. The only differences noted were in size, the hairs from the initials being larger than any other hairs in the bottom of the conceptacle, and an occasional tendency to divide laterally, thus forming a complex of cells.

There are three kinds of hairs found besides those arising from the initials: the long colorless hairs near the ostiole, the paraphyses on which antheridia are sometimes borne, and short hairs. The last are seen also in hair pits, and are found in the young stage of conceptacles but not in the mature stage. Paraphyses are not numerous and will be discussed later in connection with antheridia. None of the hairs are present in great numbers; in fact, *Pelvetia* is not a hairy plant, and this makes it the more surprising that hair pits are found.

The conceptacles located lower down on the fronds are full of cells, and it is concluded that they started as conceptacles but developed vegetatively, although they may have functioned as conceptacles and then filled up. Of course they are older in the life history, because conceptacles originate only at a growing point.

SEX ORGANS.—Oogonium initials appear early in the development of the conceptacle, and can be recognized by their greater breadth as compared with other initials. They divide into two cells, the lower of which becomes the stalk cell; the upper develops into the oogonium (fig. 10), containing at first a single nucleus which divides while the oogonium cell is still slender, but has elongated considerably (fig. 11). The oogonium then swells and becomes oval and the next division occurs, producing four nuclei which lie grouped in the center of the oogonium (fig. 12). The oogonium then increases

to its full size; the four nuclei scatter (fig. 13), and then divide again, producing the final number of eight (fig. 14). Reduction of the chromosome number takes place in the first two divisions. The mature oogonium is surrounded by a wall of three layers, the exochiton, mesochiton, and endochiton.



FIGS. 10–18.—Fig. 10, oogonium in uninucleate stage with stalk cell; fig. 11, oogonium in which nucleus has divided; fig. 12, 4 nuclei grouped in center of oogonium; fig. 13, 4 nuclei scattered; fig. 14, third division taken place; fig. 15, 4 oospheres formed in oogonium, one abortive nucleus extruded between eggs; fig. 16, oogonium with 4 eggs; fig. 17, oblique division into 2 eggs, 6 abortive nuclei; fig. 18, longitudinal division into 2 eggs (×415).

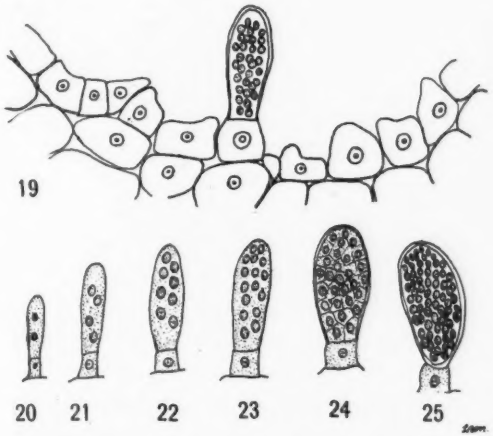
The next step is the division of the contents of the mature oogonium into eggs. The European species produces two eggs, whereas all of the material which the writer first examined produced four eggs instead of two (figs. 15, 16). This was the material collected at Redondo. Other material examined later was found to produce

in part the usual number for the European species, two eggs in each oogonium. The division was not transverse, however, but usually oblique (fig. 17) and sometimes longitudinal (fig. 18). The eggs contained one nucleus each for the most part, although binucleate eggs were found. The remaining six nuclei abort and are extruded into the mucilage which occupies the space between the eggs (figs. 15–18). The oogonia and the eggs are quite large, are very numerous in the conceptacles, and are scattered over the entire inner surface.

The antheridia are even more numerous than the oogonia, and are likewise found in all parts of the conceptacle except in the ostiole. They differ much in appearance from the antheridia of *Fucus*, because they do not as a rule grow upon paraphyses, but are produced upon a single-celled stalk just as the oogonia are, or upon a stalk of two cells. They are sometimes found branching from paraphyses, however, although this condition is not common. The antheridium initial buds from a cell of the floor of the conceptacle much as the oogonia do, except that when an oogonium is to be formed the whole upper surface of the cell becomes convex and papillate; whereas, when an antheridium is to be formed, the papillation appears at one side of the upper surface of the floor cell (fig. 19). A wall forms and cuts off the antheridial from the basal cell, following which the nucleus of the antheridial cell divides; and it now appears as a very slender, hairlike cell with two nuclei, one above the other (fig. 20). The next division forms four nuclei and reduces the chromosome number (fig. 21). The cell elongates and the four nuclei divide into eight (fig. 22). The next division produces 16 nuclei, and the antheridial cell has attained its full length (fig. 23), but grows laterally and becomes about half as wide as long. At the 32-nucleate stage evanescent walls appear (fig. 24), reminiscent of the cellular antheridia of the more primitive members of the Phaeophyceae, as *Ectocarpus*, *Cutleria*, and *Zanardinia*. The final division results in 64 nuclei (fig. 25). The ephemeral segmentation disappears in this stage, and the 64 nuclei and later the 64 sperms are free in the antheridial cavity.

The antheridium is surrounded by two walls, an inner and an outer, which separate in later stages and are readily seen. When the sperms leave the conceptacle cavity they go inclosed in this

wall. The inner wall is oval when freed from the outer, being smaller at its lower end. Antheridia were seen detached from the stalk and lying in the ostiole.



FIGS. 19–25.—Fig. 19, antheridium initials in floor of conceptacle; fig. 20, antheridium with 2 nuclei and stalk cell; fig. 21, antheridium with 4 free nuclei; fig. 22, 8-nucleate stage of antheridium; fig. 23, stage with 16 nuclei; fig. 24, 32 nuclei with delicate walls separating them; fig. 25, antheridium with 64 nuclei which will become sperms (×843).

Discussion

The apical cell of *Pelvetia fastigiata* is the typical pyramidal apical cell found in the Fucaceae, and has been described by OLTMANNS (12) for *P. canaliculata* and by HOLTZ (6) for *P. fastigiata*. The structure of the thallus is more simple than that of most Fucaceae, and hyphae are lacking except at the base of the stele.

The filaments forming the web of the central cavity are composed of pith cells, and their walls are formed of a great number of lamellae instead of three as LE TOUZÉ (9) believed. LE TOUZÉ found the outer wall to be composed of pectocellulose and the inner wall of pectin. The lamellae are not mentioned elsewhere in the literature.

The walls of cells of the pith and cortex are perforated and there is protoplasmic connection (figs. 2, 3). LE TOUZÉ denied this but

OLTMANNS (13) thinks that it is probable. All pith cells have nuclei, although LE TOUZÉ maintains that they have none. He worked on the histology of several members of the Fucaceae.

The literature up to date classes *Pelvetia* among the genera of the Fucaceae having no hair pits. However, the writer found that hair pits exist in *Pelvetia fastigiata* (fig. 4). OLTMANNS (13) states: "Fasergrübchen dürften fehlen bei *Pelvetia*." HOLTZ (6) states that conceptacles were scattered over the entire plant rather sparsely, and the writer thinks that the scattered pits found by HOLTZ were hair pits, and not conceptacles.

NORDHAUSEN (11) describes three types of hairs in conceptacles: the long colorless ones near the mouth, the branching paraphyses on which antheridia are borne, and short brown hairs. All three are found in *Pelvetia*. The short hairs seem peculiar to the immature stage and are very often found in hair pits. NORDHAUSEN believes that they crowd out the long hairs of hair pits.

Pelvetia has always been reported as having two eggs, except by GARDNER (5), who maintained as rare a condition in which three eggs occurred. His drawing shows the same appearance as the four-egg condition uniform for the first material investigated in this study, and occurring also in the other material (fig. 15). This number brings *Pelvetia* nearer to the *Fucus* type.

In *Pelvetia canaliculata* the contents of the oogonia divide in a transverse plane to produce two eggs, and the abortive nuclei are extruded at the equator, where they remain visible in the mucilage and finally disintegrate. HOLTZ (6) described *P. fastigiata* in the same way, while GARDNER (5) described the division as longitudinal, or sometimes oblique, and the extrusion of the abortive nuclei as occurring between the eggs. The writer has confirmed in part the views of GARDNER on this point. The division is generally oblique (fig. 17), often longitudinal when two eggs are formed (fig. 18). Only in one case out of hundreds was a transverse division found. When four eggs are formed they are arranged in tetrads. The region between the eggs is filled with mucilage in which the abortive nuclei lie.

Nowhere in the literature has mention been made of the mode of attachment of antheridia in the conceptacle in *P. fastigiata*. In-

stead of being on branched paraphyses they are borne on stalk cells like oogonia (fig. 25).

The conceptacle develops from two epidermal initials and its walls are formed from their basal segments (fig. 5). The mouth is walled by epidermal cells. The genetic layer is therefore derived entirely from the basal segments of the two initial cells. No tongue cells, such as SIMONS (15) found for *Sargassum*, are produced in *Pelvetia fastigiata*, and the basal segments are cubical instead of cup-shaped. The cortex does not enter into the conceptacle. HOLTZ believed that there was extensive disintegration in epidermal and basal cells, and that the cortex entered into conceptacle formation. BOWER (2) also believed that the cortex cells helped to form the genetic layer. HOLTZ' conclusions have been refuted by NIENBURG (10).

Summary

1. The cell walls of the pith and cortex of *Pelvetia fastigiata* are perforated, and protoplasmic connections are evident.

2. Walls of pith cells are formed of many lamellae.

3. Hair pits exist, although not in great numbers.

4. Conceptacles are found below the fruiting tips. The lower conceptacles have no sex organs.

5. There are often four eggs in each oogonium instead of two, as usually reported. Division is not transverse but longitudinal. Binucleate eggs occur.

6. Abortive nuclei are extruded between the two or four eggs, not in the equator and outside, as in the European species of *Pelvetia*.

7. Oogonia are stalked. Antheridia are stalked like oogonia, only seldom being borne on paraphyses. Antheridia have two walls.

I am indebted to Professor C. J. CHAMBERLAIN for suggestions and criticisms.

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STUDIES IN THE GENUS BIDENS. IX

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 388

EARL EDWARD SHERFF

(WITH PLATES XIV–XVI)

Bidens fulvescens sp. nov.—Fruticosa vel subfruticosa, caule ramisque tetragona et glabrata, demum 1.5–2.1 m. alta. Folia petiolata petiolis tenuibus usque ad 9 cm. longis, petiolo adjecto usque ad 2.2 dm. longa, pinnatim 3- vel 5-partita, juvenilia plerumque pilis numerosis fulvescentibus vel etiam ferrugineis obsita, foliolis ovato-lanceolatis vel etiam anguste oblongo-lanceolatis, apice subobtusis vel breviter acuminatis, margine serratis et ciliatis, demum plus minusve glabratis, supra valde viridibus, infra pallidioribus venulis numerosis perspicuis coloratis, basalibus plerumque petiolulatis. Capitula numerosa, corymbo-paniculata, radiata, pansa ad anthesin tantum 3.5–5 mm. alta et vix 1 cm. lata, pedicellis tenuibus, pubescentibus, saepius 0.5–2 cm. longis. Involucri bracteae exteriores circ. 5, lineares, patentes vel recurvatae, tantum 1–1.5 mm. longae, minute pubescentes vel glabratae, apice subobtusae; interiores lanceolatae 3–4 mm. longae. Flores ligulati plerumque 5, flavidi, ligula late oblanceolati, apice circ. 3-dentati, tantum circ. 5 mm. longi. Achaenia linearia, nigra, obcompressa, glabra, spiraliter per 1 vel 1.5 convolutiones torta, circ. 8–11 mm. longa et 0.6–0.8 mm. lata, exaristata sed ad apicem rariter 1 vel 2 minutis setis munita.

Otto Degener 2515, in open woods, north slope of Mt. Kaala, Isl. of Oahu, Hawaiian Isls., February 11, 1928 (Hb. Field Mus., 3 type sheets).

Bidens wiebkei sp. nov.—Erecta, glabra, infra demum fruticosa supra herbacea, usque ad circ. 1 m. alta, ramis acriter tetragonis et siccis plus minusve purpurascentibus. Folia petiolata petiolis tenuibus plerumque 2–4.5 cm. longis, petiolo adjecto usque ad 1.3 dm. longa, 3- vel 5-partita, foliolis membranaceis lanceolatis, acriter serratis, imis 5-partitorum saepe breviter petiolulatis, terminali moderate acuminato. Capitula numerosa in inflorescentia corymboidea, tenuiter pedicellata pedicellis fere glabris, radiata, pansa ad

anthesin 1–2 cm. lata et circ. 4.5–6 mm. alta. Involucri bracteae exteriores 4–6, patentes vel suberectae, lineares, apice subobtusae, tergo saepe hispidulae, 2–3 mm. longae; interiores lanceolatae, plerumque 3–4.5 mm. longae. Flores ligulati 4–6, flavidi, ligula oblongi vel late elliptico-oblanceolati, apice circ. 3-denticulati, 5–9 mm. longi. Achaenia demum nigra, valde obcompressa, curvata vel saepius etiam per 1–2.5 convolutiones torta, marginata vel alata, marginibus glabris vel sparsissime erecto-setosis, nunc multo infra nunc ad corporis apicem in 1 vel 2 filiformes, remote retrorsumque hamosas usque ad 1.5 mm. longas aristas productis vel saepius aristis deficientibus, corpore faciebus glabro vel sparsim erecto-setoso, nitido, 6–8 mm. longo et 0.9–1.2 mm. lato.

Otto Degener and *Henry Wiebke* 3005, in scrub vegetation, upper part of Halawaiki Gulch, Isl. Molokai, Hawaiian Isls., June 21, 1928 (3 type sheets, Hb. Field Mus.: cotypes, Hb. Berl.; Hb. Boiss.; Hb. Brit. Mus.; Hb. Mun.; Hb. Mus. Vienna; Hb. N.Y. Bot. Gard.). Pl. XIV.

A species allied to *Bidens sandvicensis* Less., a species unknown in its normal state except from the Islands of Kauai, Oahu, Maui, and Hawaii. *B. sandvicensis* var. *caduca* Sherff was originally described by HILLEBRAND from Molokai, without stating the exact locality. Apparently the var. *caduca* is widely different from *B. wiebkei* in having shallower "serratures and cusps, ... achenes not margined . . . ." and in having achenes straight or only lightly curved. Some of the many specimens of *B. wiebkei* examined have the achenes nearly all exaristate, but a few aristate with one or two aristae at the very apex. Others have 1 or 2 aristae rather commonly, these coming out about one-fifth to one-third the way down the sides, and being continuous with the conspicuously flattened achenial margins. The heads are more numerous than in *B. sandvicensis* proper, and when in fruit differ noticeably because of the more or less twisted and bent achenes.

The species is named in honor of *Mr. Henry Wiebke*, who, in collaboration with *Professor Degener* of the University of Hawaii, has done much valuable work in collecting the native Hawaiian plants for taxonomic study.

Bidens coartata sp. nov.—Perennis, infra fruticosa, ± 5 dm. alta, ramosa, caule ramisque tetragonis, glabris. Folia petiolata petiolis

tenuibus usque ad 6 cm. longis, petiolo adjecto usque ad 1.5 dm. longa, pinnatim 3–5-partita, foliolis ovatis vel lanceolatis, membranaceis, acriter dentibus calloso-apiculatis serratis, glabris ac eciliatis, imis saepe ad marginem inferiorem plus minusve irregulariter divisis, terminali apice breviter acuminato. Capitula subnumerosa et saepe in inflorescentia densa subcorymboideaque coartata, radiata, pansa ad anthesin circ. 1.5 cm. lata et 6–8 mm. alta. Involucri bracteae glabrae vel glabratae, exteriores circ. 5–7, lineares, apice subobtusae, circ. 3 mm. longae; interiores lanceolatae paulo longiores. Flores ligulati plerumque 5, flavidi, ligula late oblanceolato-elliptici, apice subintegri, circ. 7–10 mm. longi. Achaenia anguste linearia, nigra, exalata, plana, recta vel moderate torta, faciebus glabrata et leviter paucistriata, marginibus sparsim suberecto-hispida, apice aegre spinulosa sed exaristata, corpore 7–10 mm. longa et 0.6–1 mm. lata, demum paleas manifeste superantia.

Otto Degener 2677*b*, sunny slopes from Woodlawn along east rim of Manoa Valley toward Mt. Olympus, Isl. of Oahu, Hawaiian Isls., February 28, 1928 (2 type sheets, Hb. Field Mus.: cotypes, Hb. Berl.; Hb. Brit. Mus.; Hb. N.Y. Bot. Gard.); *idem* 2676, two-thirds the way up Mt. Olympus by way of Pauoa Flats, Isl. Oahu, February 25, 1928 (Hb. Brit. Mus.; Hb. Field Mus.; Hb. N.Y. Bot. Gard.).

Bidens salicoides sp. nov.—Fruticosa, erecta, glabra, ramosa, ±6 dm. alta, caule ramisque plus minusve tetragonis. Folia petiolata petiolis tenuibus usque ad 5 cm. longis, petiolo adjecto usque ad circ. 1.5 dm. longa, principalia pinnatim sed saepe irregulariter 2–5-partita, foliolis moderate vel late linearibus ac circumambitu foliis non nullorum specierum *Salicis* nonnihil similibus, integris vel interdum paucidentatis, marginibus saepe parce revolutis, membranaceis, terminali usque ad 8 cm. longo et 12 mm. lato, lateralibus plerumque minoribus et sessilibus vel imis subpetiolulatis; summa nunc indivisa, nunc ternata. Capitula subcorymboidea, radiata, pansa ad anthesin ±1.5 cm. lata et circ. 6–8 mm. alta, graciliter pedicellata pedicellis 1–4 cm. longis. Involucrum glabratum, bracteis exterioribus circ. 6–7, anguste linearibus, apice calloso subacutis, costa mediana atris, circ. 3–4 mm. longis, quam interioribus lanceolato-ovatis paulo brevioribus. Flores ligulati 3 vel 4, forsitan interdum 5, flavidi, lineari-elliptici, apice obsolete denticulati, 1.2–1.4 cm.

longi. Achaenia linearia, plana, plumbeo-atra, exalata, recta vel moderate torta, plerumque glabra, faciebus obscure sulcata (omnino circ. 16 sulcis), corpore 7–10 mm. longa, apice ipso nunc 1- vel 2-aristata aristis nudis usque ad 1 mm. longis, nunc calvis.

Henry Wiebke 3084, arid region, East Ohia, Isl. Molokai, July 17, 1928 (Hb. Field Mus., 3 type sheets: cotypes, Hb. Berl.; Hb. N.Y. Bot. Gard.). Pl. XV.

The five specimens studied had come from a single plant, the only one found. Professor DEGENER, to whose kindness I am indebted for the privilege of examining the material, had suspected that this might be "HILLEBRAND'S lost variety"; i.e., *Campylotheca sandvicensis* var. *β*, Hillebr. Fl. Hawaiian Isls. 214. 1888. HILLEBRAND'S plant, renamed by me *Bidens sandvicensis* var. *caduca* (BOT. GAZ. **85**:7. 1928), came from an unknown locality on the Island of Molokai and was described in terms that might indeed apply to our plant. However, from a study of HILLEBRAND'S various determinations of Hawaiian Island *Bidens* material to be found at Gray Herbarium and in the British Museum of Natural History, it is difficult to believe that HILLEBRAND would have referred this plant to the species *sandvicensis*. Apparently the identity of his variety must remain obscure until an authentic specimen labeled by him can be located.

Since the present plant is clearly separate from *B. sandvicensis* and requires a distinct specific name, and since it is impossible to utilize the name *caduca*, I have chosen *salicoides*. This name is suggested by the illusory resemblance of the herbarium specimens to specimens of various species of *Salix*, a resemblance arising from the *salicoid* outline of the simple leaves or, in the case of the much more numerous compound leaves, of the leaflets.

Bidens populifolia sp. nov.—Herba e radice (ut videtur) annua, usque ad 8dm. alta, glabra, caule tetragono, subsimplici vel etiam valde ramoso, non robusto. Folia petiolata petiolis tenuibus nunc 1.5–6 cm. nunc 6–10 cm. longis, petiolo adjecto usque ad 2 dm. longa, membranacea, pallidiora, vix ciliata, serrata dentibus rotundatis obtuseque calloso-apiculatis, plerumque indivisa, circumambitu ovato-cordata vel deltoideo-cordata, basi saepe tantum subcordata vel etiam truncata obliquave, apice nunc subobtusa nunc acuta vel

rarius plus minusve subacuminata; rariter (et plus minusve irregulariter) pauca 2–3-partita, foliolo terminali late rhomboideo- lanceolato vel ovato-lanceolato vel rotundato, lateralibus obliquis et rhomboideo-ovatis. Capitula non numerosa, in inflorescentia corymbiformi disposita, radiata, pedicellata pedicellis tenuibus usque ad 5 cm. longis, pansa ad anthesin circ. 3 cm. lata et 7–9 mm. alta. Involucri bracteae valde dissimiles, exteriores 5–7, patenti-reflexae, glabrae, nunc late oblongo-lineares nunc spathulato-obovatae apice obtusae vel rotundatae, 1.5–3.5 mm. longae; interiores lanceolatae, apicem versus minute pubescentes, 5–6.5 mm. longae. Flores ligulati 6–8 (rariter tantum 5), flavidi, ligula oblongo-lineares vel oblanceolati, apice plerumque 3-dentati, 1.2–1.5 cm. longi. Achaenia anguste linearia, recta, exalata, subnigra, obcompressa, unica facie obsolete circ. 8-striata, margine sparsim erecteque ciliata, corpore 7–12 mm. longa et 0.8–1.1 mm. lata et paleas apice rufescentes paulo demum saepe excedentia, apice plerumque biaristata aristis tenuibus, supra retrorsum infra antrorsum hamosis, usque ad 1 mm. longis.

Otto Degener, Ross S. Bean, D. Le Roy Topping, and *Anthony Apo* 2514, growing with *Pandanus* and stunted *Metrosideros,* grassy slope back of small Hawaiian church on east side of Kahana Valley, Isl. Oahu, Hawaiian Isls., February 12, 1928 (Hb. Field Mus., 4 type sheets: cotypes, Hb. Berl.; Hb. Boiss.; Hb. Brit. Mus.; Hb. Gray; Hb. Kew; Hb. Mo. Bot. Gard.; Hb. Mun.; Hb. Mus. Vienna, etc.); *Ross S. Bean* 2322, sunny, rocky ridge, foot of left ridge of Kahana Valley, Isl. Oahu, Hawaiian Isls., January 1, 1928 (Hb. Field Mus.; Hb. Kew). Pl. XVI.

This species is characterized by the very distinct *Populus* aspect of its foliage, the resemblance to the foliage of *P. deltoides* Marsh, for example, being at times very striking. HILLEBRAND (Fl. Hawaii. Isls. 215. 1888) appears not to have known this plant. Under *Campylotheca macrocarpa* he lists the (to *B. populifolia*) faintly similar var. *ovatifolia,* transferred by him from varietal rank under *Bidens sandvicensis* Less., where ASA GRAY had originally placed it. He then gives a detailed description of mature plants, a description which could not have come from the single, very immature type of the var. *ovatifolia* (U.S.). The true var. *ovatifolia,* recently placed by me (BOT. GAZ. **85**:7. 1928) under *Bidens macrocarpa,* has sharply

serrate leaves, while *B. populifolia* has round and obtuse teeth. It is clear from HILLEBRAND's text that, the synonym var. *ovatifolia* Gray being excluded, his treatment applied to the herbaceous forms of *Bidens asymmetrica* (Lévl.) Sherff, found upon Oahu. In fact, we may note that one able student of the Hawaiian flora, JOSEPH ROCK, had labeled a cotype specimen of *B. asymmetrica* (*Faurie* 960, Par.) with the equation: "*Coreopsis macrocarpa* Hbd. var. β Hbd. = *Lipochaeta asymmetrica* Lévl. (teste) Rock." (the latter name being a synonym for *Bidens asymmetrica*). A consideration of these herbaceous forms of *B. asymmetrica* ("Achenes . . . . often spirally twisted"), however, shows that they too have little in common with the species here named *B. populifolia*.[1]

BIDENS MAUIENSIS **cuneatoides** var. nov.—A specie foliis nunc indivisis nunc tripartitis laminis segmentisve lanceolatis vel ovatis vel subrhomboideis vel etiam late cuneato-spathulatis, habitu *Bidenti cuneatae* non dissimilis differt.

Otto Degener and *Henry Wiebke* 2261, arid aeolian deposits southeast of Wailuku, Isl. of Maui, Hawaiian Isls., Jul. 7, 1927 (Hb. Berl.; Hb. Boiss.; Hb. Field Mus., 2 sheets; Kew; Mun.); *iidem* 2680, on barren, aeolian deposits near Wailuku, Isl. of Maui, Hawaiian Isls., July 9, 1927 (type in Hb. Field Mus.: cotypes in Hb. Berl.; Hb. Boiss.; Hb. Brit. Mus.; Hb. Kew; Hb. N.Y. Bot. Gard.); *H. Mann* and *W. T. Brigham* 372, Isthmus of Maui (Hb. Bish. Mus.; Hb. Brit. Mus.; Hb. Cornell Univ.; Hb. Deless.; Hb. Field Mus., 2 sheets; Hb. Gray; Hb. Kew; Hb. Mo. Bot. Gard., etc.).

The type material of *Bidens mauiensis* (*United States Southern Pacific Exploring Expedition* under *Capt. Wilkes*, sandy or dry hills near the coast, Island of Maui, Hawaiian Islands, 1838–1842; Hb. Gray) had the leaves, except for one small branchlet apparently somewhat ignored by ASA GRAY in writing his original description, rather well dissected, with linear segments. The duplicate sheet in the Torrey Herbarium (Hb. N.Y. Bot. Gard.) has, at the right, one specimen with some leaves tripartite and some undivided, the blades or their segments more or less rhombic-ovate and somewhat suggestive of those of *Bidens cuneata* Sherff. Doubtless had GRAY seen this last specimen he would have presented a different or an addition-

[1] It is a pleasure to acknowledge my indebtedness to *Messrs. Bean* and *Degener*, also their associates, for making special trips to the type locality and securing a large representation of material for my study.

al treatment. In any case, we are fortunate in having at hand two new and excellent series of specimens collected in July, 1927, by *Degener* and *Wiebke* in the type region. Nos. 2678 and 2679 by these collectors are of the species as described by GRAY. Their nos. 2261 and 2680 have the leaves simple or merely tripartite. These latter thus match the ignored or at least undescribed specimens collected together with GRAY's type of the species proper. They are matched in turn by *Mann* and *Brigham* 372. The specimens of this last number at Delessert Herbarium and at Bishop Museum have the leaves mostly simple, but elsewhere they are mostly tripartite.

The pronounced difference between the two types of foliage makes it appear worth while to treat the plants with the simple or tripartite leaves as varietally distinct.

BIDENS MAUIENSIS **forbesiana** var. nov.—Ex characteribus maximam partem var. *lanaiensi* similis sed foliis plerumque indivisis rarius tripartitis differt.

G. C. Munro 451, Maunalei, Isl. of Lanai, Hawaiian Isls., April 19, 1915 (type in Hb. Bish. Mus.).[2]

This and the next following were included by me in a former paper (BOT. GAZ. **80**:381. 1925) among the plants referred to *B. mauiensis* var. *lanaiensis*. The considerations already referred to, however, for the var. *cuneatoides* make it appear similarly preferable here to segregate from the var. *lanaiensis* the two types of foliage which *differ in not being finely dissected.*

The name *forbesiana* alludes to Professor CHARLES N. FORBES, who, before his death, had studied the type plant and regarded it as typifying a new variety.

BIDENS MAUIENSIS **media** var. nov.—Ex characteribus maximam partem var. *lanaiensi* similis sed foliis minus decomposita; a var. *forbesiana* foliis non plerumque indivisis differt.

G. C. Munro 450, Maunalei, Isl. of Lanai, Hawaiian Isls., April 19, 1915 (type in Hb. Field Mus.: cotype in Hb. Bish. Mus.).

[2] In view of the present comparative inaccessibility of much of the material in the Bishop Museum (Honolulu), it may be remarked that this and some 2000 other important specimens of *Bidens, Coreopsis, Cosmos, Isostigma, Taraxacum, Xanthium,* and Compositae generally are represented by the writer's photographs, a duplicate set of which is in the Herbarium of Field Museum of Natural History (Chicago).

BIDENS BIPINNATA L. Sp. Pl. 832. 1753; *B. fervida* Nocca *in* Fischer Cat. Gorenki edit. II, 37. 1812 (*nomen*); *B. myrrhidifolia* Tausch, Flora **19**:394. 1836; *B. cicutaefolia* Tausch, *loc. cit.* 395; *B. elongata* Tausch, *loc. cit.* (*ex descript. etc.*); *B. tenuifolia* Tausch, *loc. cit.*; *B. decomposita* Wall. *ex* DC. Prodr. **5**:602. 1836; *Coreopsis corymbifolia* Ham. *in* Wall. *ex* DC.[3] *loc. cit.*; *Bidens pilosa* var. *decomposita* (Wall. *ex* DC.) J. D. Hook. Fl. Brit. Ind. **3**:309. 1881; *B. kotschyi* Schz. Bip. *ex* Walp. Rep. **6**:168. 1846; *B. kotschiana* Schz. Bip. *loc. cit.*; *Kerneria bipinnata* (L.) Godr. et Gren. Fl. Fr. **2**:169. 1850; *B. pilosa* var. *bipinnata* (L.) J. D. Hook. *loc. cit.*; *B. pilosa* var. *decomposita* (Wall. *ex* DC.) J. D. Hook. *loc. cit.*[4]

FISCHER (*loc. cit.*) cited "*B. fervida* Nocoa," which of course was erroneous as to the spelling of NOCCA's name. With FISCHER, *B. fervida* amounts to a name (*nomen*) only. In NOCCA's Synonimia Plant. Hort. Bot. Ticinensis (1804), *B. fervida* is not given. NOCCA's many references there to LAMARCK's Encyclopaedia, however, show that he was entirely familiar with that work, and it was in that work that *Bidens fervida* Lam. was published. The real *B. fervida* Lam. was *Spilanthes oleracea* L. and is generally regarded as such (*cf.* Moore, Proc. Amer. Acad. **42**:530. 1907). NOCCA's material does not appear to have become widely distributed in European herbaria, but the Ledebour Herbarium (Hb. Petrop.) has an old folder with two unmounted specimens which are without doubt authentic. They are in fruit and in excellent condition. Both are *B. bipinnata* L.

TAUSCH (*loc. cit.*) had sought to distinguish among five forms cultivated in gardens under the name *B. bipinnata*. He described each form separately, retaining one as *B. bipinnata* and creating new names for the other four. *B. cicutaefolia* Tausch and *B. myrrhidifolia* Tausch each are represented by one of his own cultivated specimens (Hb. Univ. Leips.) and are seen to be purely *B. bipinnata*.[5]

[3] DECANDOLLE cited Wallich's Catalogue, but the name there given is "*Coreopsis ? corindifolia Ham.*"

[4] It being my intention at this time only to discuss the status of TAUSCH'S proposed names and to place on record certain scattered but important observations, no attempt is made here to present an exhaustive synonymy of *B. bipinnata* or to discuss all of the synonyms given.

[5] TAUSCH unfortunately cited as a synonym for his *B. cicutaefolia* the name *Chrysanthemum chinense*, etc., Plukenet Phytograph, pl. 22, fig. 4, 1691. PLUKENET'S plant, however, was *B. chinense* (L.) Willd., as stated by me in a former paper (BOT.

The meager description of *B. tenuifolia* Tausch matches *B. bipinnata* as far as it goes. A plate cited by TAUSCH, "*Chrysanthemum cannabinum etc.* Moris. Hist. 3^{VI}: tab. 7, fig. 23," is of *B. bipinnata* and is so treated by DECANDOLLE (Prodr. **5**:603. 1836) and O. E. SCHULZ (Engler Bot. Jahrb. **50**, Suppl.:183. 1914). At Leipsic I sought, in 1924, to find further evidence in the form of authentic specimens of *B. tenuifolia* Tausch, but failed entirely.

B. elongata Tausch appears likewise unrepresented by an authentic specimen today, but the description given by TAUSCH, as also his context, leaves no doubt as to the identity of *B. elongata* with *B. bipinnata*.

HOOKER (*loc. cit.*) treated *B. pilosa* L. as being made up of three varieties, viz. *pilosa* proper, *bipinnata*, and *decomposita*. Taxonomists very generally, however, have refused to accept such treatment. *B. pilosa* and *B. bipinnata*, while sometimes approaching each other, are usually quite different and easily distinguished.

B. decomposita Wall. *ex* DC. was founded upon WALLICH'S Catalogue no. 3188 (DC. distrib. no. 298). A fine specimen of this number, from KUNTH'S herbarium, is still extant (Hb. Berl.). This is purely *B. bipinnata* L., and, in fact, had been so determined in 1911 by O. E. SCHULZ. Similarly other specimens of this number (Hb. Prodr. in Hb. Deless.; Hb. Kew) are either normal *B. bipinnata* or represent slight and insignificant variations from *B. bipinnata*.

BIDENS PILOSA var. MINOR (Bl.) Sherff, Bot. Gaz. **80**:387. 1925; *Coreopsis leucorrhiza* Lour. Fl. Coch. edit. I:508. 1790 (*ex descript. et patria*); *C. leucorhiza* Lour. *loc. cit.* edit. II:622. 1793 (*ex descript. et patria*); *Bidens hispida* H. B. K. Nov. Gen. et Sp. **4**:186 (237). 1820; *B.? leucorhiza* (Lour.) DC. Prodr. **5**:605. 1836; *B. andicola* var. *β*, Wedd. Chlor. And. **1**:70. 1855 (*ex synon.* H. B. K.).—DECANDOLLE (*loc. cit.*) treated LOUREIRO'S *Coreopsis leucorrhiza* with misgivings, placing it under *Bidens* with an interrogation and listing it among the species not well enough known. FORBES and HEMSLEY (Jour. Linn. Soc. **23**:435. 1888) declared LOUREIRO'S plant to have

GAZ. **61**:499. 1916). A consideration of TAUSCH'S entire context, much of which was inaccessible when writing the former paper, shows that TAUSCH'S name *B. cicutaefolia* must rest upon the real plants studied by him, also their published description, and that the reference to PLUKENET must be ignored.

been "insufficiently described for recognition."[6] Reference to LOUREIRO's rather full description shows that the plant described grew in fields near Canton, China. The leaves were 5-partite, their leaflets lanceolate. The flowers were yellow and the heads had 6 rays, these neuter and with ovate ligules. The achenes were 3-aristate, with the aristae retrorsely barbed. There is only one *Bidens* known in the vicinity of Canton which fits this description. That is *B. pilosa* var. *minor* (Bl.) Sherff.[7] In certain herbaria are a number of specimens from places near to the type locality (Hongkong, Hainan, etc.), among them the following:

Hance 299, Hongkong (Hb. Gray); *Dr. Aug. Henry* 75, Hongkong (Hb. Mo. Bot. Gard.); *idem* 8769, Isl. of Hainan, November, 1889 (Hb. Gray); *C. O. Levine* 278, Isl. of Honam, Kwang Tung Province, October 1, 1917 (Hb. Mo. Bot. Gard.); *C. Wright*, Hongkong, 1853–1858 (Hb. Gray).

In a former article (BOT. GAZ. **61**:497. 1916), I stated the reasons for reducing *B. hispida* H.B.K. to synonymy under *B. pilosa* L. The type of *B. hispida* (Hb. Par.) had been described as having the florets not yet open. At the former writing I had been impressed with the fact that several more mature specimens matching the type of *B. hispida* had been collected by various botanists, and that these tended to have minute, yellowish-white rays. In 1924 it was my good fortune to find a better developed cotype specimen in the Willdenow Herbarium, no. 15022, fol. 3 (Hb. Berl.). The one small flowering head had three or four minute rays and thus revealed definitely the identity of *B. hispida* with the minutely radiate form of *B. pilosa* that passes as var. *minor*.

BIDENS PILOSA L. Sp. Pl. 832. 1753; *B. decussata* Pav. *ex* DC. Prodr. **5**:599. 1836.—In the Prodromus Herbarium (Hb. Deless.) are five sheets to represent *B. hispida* H.B.K. These all bear only ordinary *B. pilosa* L. One has an old label saying "*B. decussata* Perou Pavon." and, being clearly the basis of the name *B. decussata* Pav. *ex* DC., enables us at once to reduce this name to synonymy with *B. pilosa* L.

[6] For many data regarding LOUREIRO and his work, *vide* American Botanist **25**: 129. 1919; *ibid.* **26**: 28. 1920.

[7] *B. pilosa* var. *radiata* Schz. Bip. is found in that vicinity but its rays are more of a whitish or yellowish-white color, not a pure yellow as described by LOUREIRO.

B. DURANGINENSIS Sherff, BOT. GAZ. 70:90, Pl. XI. 1920; *B. anthriscoides* var. *angustiloba* DC. Prodr. 5:601. 1836.—DECANDOLLE founded his *B. anthriscoides* var. *angustiloba* upon *Berlandier* 875, collected at the City of Mexico. His type is still extant (Hb. Deless.) and is found to be inseparable from *B. duranginensis*, a species much closer to *B. pilosa* L. than to *B. anthriscoides* DC.

Bidens musoziana nom. nov.; *Coreopsis arenicola* S. L. Moore, Jour. Linn. Soc. 37:170. 1905; *Bidens arenicola* (S. L. Moore) Sherff, BOT. GAZ. 59:309. 1915; *non* Gandoger Fl. Lyon. 122. 1875.—This species is here renamed[8] to avoid any possible confusion with the ill-advised name *B. arenicola* Gand. The first cited specimen of GANDOGER'S species was one from Arnas (Rhône), France. GANDOGER cited also the synonym *B. arenaria* of his own mss. In herbaria we have at least four good specimens of GANDOGER'S own collecting in the vicinity of Lyons and labeled by him *B. arenaria* Gdgr. One, without a number, was collected at Arnas, Rhône, September, 1872 (Hb. Mus. Vienna). A second and third, *Gandoger* 967, were collected at Arnas, August 11, 1869 (Hb. Mo. Bot. Gard.; Hb. Univ. Vienna). A fourth, *Gandoger* 599, Anse, Rhône, was collected August 6, 1866 (Hb. Kew). These all are merely simple-leaved forms of *B. tripartita* L.

BIDENS CERNUA L. Sp. Pl. 832. 1753; *B. cusickii* Greene, Pittonia 4:259. 1901.—In a former article (BOT. GAZ. 61:503. 1916), I discussed the large number of field forms which E. L. GREENE had segregated from *B. cernua* L. Some of these were reduced at that time to synonymy with *B. cernua*. *B. cusickii*, however, was left untreated until further specimens could be examined.

B. cusickii was founded by GREENE upon *Wm. C. Cusick* 1768, tules of Grande Ronde Valley, Oregon, August, 1897. Specimens of the type number are in Field Museum and at Kew. These both are slightly atypic for *B. cernua* and suggest very faintly an approach toward *B. laevis* (L.) B.S.P. On the whole, however, both the foliage and the floral characters are too close to those of *B. cernua* to permit any segregation as a distinct species.

[8] The type was collected at Musozi, Uganda Protectorate. The name was spelled by the collector, *A. G. Bagshawe*, Nuszi. By the Century Atlas (259, pl. 112. 1911) it is spelled Msozi. SPENCER MOORE'S spelling is the one adopted here.

BIDENS PILOSA var. ALAUSENSIS (H.B.K.) Sherff, BOT. GAZ. **81**: 35. 1926; *B. diversifolia* Willd. *ex* DC. Prodr. **5**:602. 1836.—WILLDENOW's original sheet of his own *B. diversifolia* is in the Willdenow Herbarium (Hb. Berl.). It bears two specimens. One is referable to *B. pilosa* var. *alausensis* (H.B.K.) Sherff, but the other appears to belong to *B. andicola* H.B.K. It was probably because of this very unlikeness between the two specimens that WILLDENOW used the name *diversifolia*. The case is evidently one of improper commixture of specimens at the time of collecting or mounting. Fortunately, the very definite status of the names *alausensis* and *andicola* permits our ignoring the name *B. diversifolia* Willd. *ex* DC.

BIDENS RADIATA Thuill. Fl. Par. edit. II: 422. 1799; *B. foliosa* Willd. Enum. Hort. Berol. Suppl. 56. 1813 (*nomen subnudum*); *etiam* Loudon Hort. Brit. 335. 1830.—WILLDENOW'S *B. foliosa* was listed as an "annual. Growing always in the open." It was listed later by LINK (Enum. Pl. Hort. Berol. 305. 1822) and by J. C. LOUDON (*loc. cit.*), the latter giving a more extended description and evidently having seen some authentic material. The type in the Willdenow Herbarium at Berlin is Hb. Willd. 15020–5. ASCHERSON, in 1869, had labeled it "*B. radiata* Thuill. fol. indivisis." A duplicate sheet in the Berlin Herbarium bears three plants, all with leaves likewise simple. The achenes on all four plants are typical for *B. radiata*, with which the name *B. foliosa* must be considered synonymous.

The reason for WILLDENOW'S name *B. foliosa* is hardly apparent, until one examines the duplicate specimen in Vienna (Hb. Mus. Vienna). This is a spray with gigantic leaves, which measure up to more than 2.1 dm. long. One leaf is tripartite, with the terminal leaflet oblong-lanceolate. The others are simple.

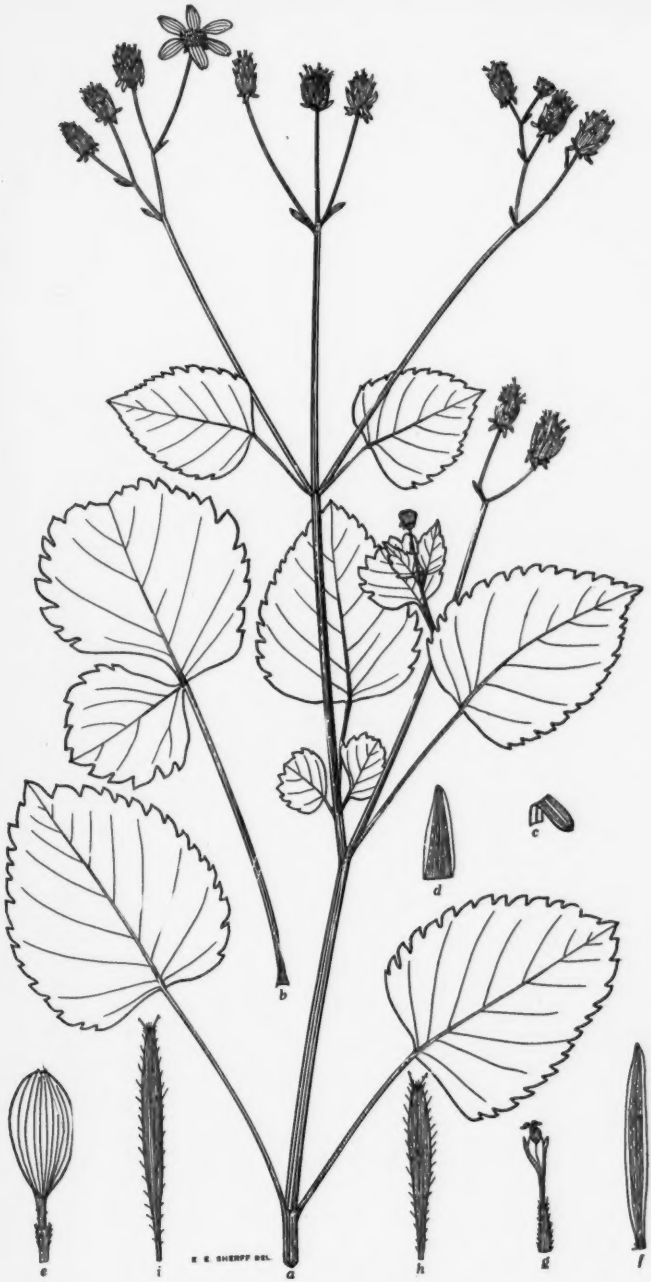
This mostly simple-leaved form of *B. radiata* is much rarer than are simple-leaved forms of the sister species, *B. tripartita* L.

BIDENS CORONATA VAR. **tenuiloba** (Gray) comb. nov.; *Coreopsis trichosperma* var. *tenuiloba* Gray Syn. Fl. Nl. Amer. **1**:295. 1884; *Bidens trichosperma* var. *tenuiloba* (Gray) Britt., Bull. Torr. Bot. Club **20**:281. 1893; *B. trichosperma* var. *tenuifolia* (Gray) Britt. *ex* Farw., Ann. Rept. Comm. Parks and Blvds. Detroit **11**:92. 1900.

Frequently, where many specimens are examined in the field, the var. *tenuiloba* is found to pass over into the species proper. The

SHERFF on BIDENS

SHERFF on BIDENS



SHERFF on BIDENS

more finely divided foliage appears to be the most pronounced diagnostic character, but even this is inconstant. Rarely the achenes are exceedingly diminutive and then a strong approach is made to *Bidens mitis* (Michx.) Sherff, as noticed by GRAY (*loc. cit.*).

FARWELL'S plant, not seen by me, was referred with an interrogation to this variety. It was described as "a peculiar form found on Belle Isle," a pleasure island at Detroit, Michigan.

CHICAGO NORMAL COLLEGE
CHICAGO, ILL.

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EXPLANATION OF PLATES XIV–XVI

PLATE XIV

Bidens wiebkei: a, flowering and fruiting branch, ×0.6; *b*, exterior involucral bract, ×3.6; *c*, interior involucral bract, ×3.6; *d*, ligulate floret, ×3; *e*, palea, ×3.6; *f*, disc floret, ×6; *g*, *h*, achenes, ×3.6; all from *Degener* and *Wiebke* 3005, cotype in Hb. Mus. Vienna.

PLATE XV

Bidens salicoides: a, flowering and fruiting branch, ×0.6; *b*, exterior involucral bract, ×3.6; *c*, interior involucral bract, ×3.6; *d*, ligulate floret, ×3; *e*, palea, ×3.6; *f*, disc floret, ×6; *g*, *h*, exterior and interior achenes, ×3.6; all from *Wiebke* 3084 (*a* from cotype in Hb. Berl. and the rest from 1st type sheet in Hb. Field Mus.).

PLATE XVI

Bidens populifolia: a, main portion of younger plant, ×0.6; *b*, leaf from lower part of same, ×0.6; *c*, exterior involucral bract, ×3.6; *d*, interior involucral bract, ×3.6; *e*, ligulate floret, ×1.8; *f*, palea, ×3.6; *g*, disc floret, ×6; *h*, *i*, achenes, ×3.6; all from *Degener*, *Bean*, *Topping*, and *Apo* 2514, cotype in Hb. Boiss.

RELATION OF PHOSPHORUS CONTENT TO SHRIVELING OF WALNUT KERNELS[1]

A. R. C. Haas and L. D. Batchelor

(with two figures)

Introduction

It is a matter of common observation that mature walnut kernels may take on various shades of amber color, and in addition show different degrees of plumpness. They may be only partially shriveled, or may be completely so, as illustrated by fig. 1. The present

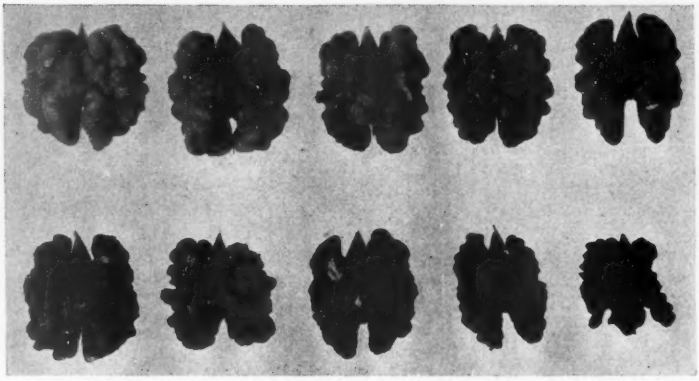

Fig. 1.—Various degrees of shriveling in walnut kernels, progressively from left to right in both rows; normal plump kernel is one extreme and "blank" kernel is the other.

standards by which walnuts are marketed are based largely on the color of the kernel and also upon the number that are free from any shriveled condition.

The walnut kernel in the early stages of development is filled with liquid, as is illustrated by fig. 2. Later gel formation takes place along the surfaces within the kernel. While this gel formation is taking place the shell becomes hardened, first at the apex and along

[1] Paper no. 191, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

the suture, and then generally throughout the entire shell. In the case of most varieties grown in southern California, the contents of the kernel have jelled considerably by late June and early July, and the entire shell has become hardened; toward the end of July or early in August the kernels have become solid. In September and October the husks should separate readily from the shell and the mature walnuts have become ready to be harvested and cured. When the nuts are graded in the packing houses some may be extremely light in weight, and may be removed by the use of air suc-

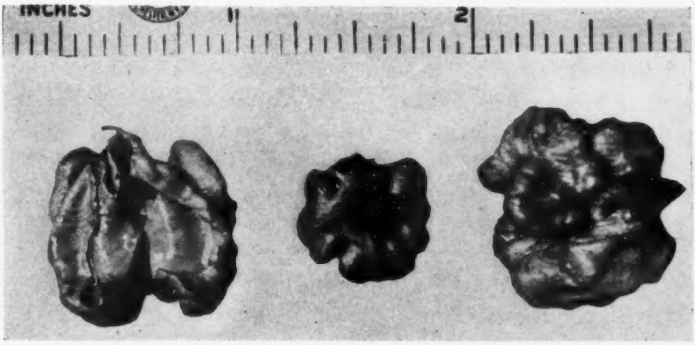

FIG. 2.—Interior and exterior view of walnut kernels when contents are still liquid

tion. The contents of such walnuts are commonly shriveled kernels or "blanks."

The view is commonly held that the shriveling of walnut kernels must take place, if at all, while the kernel is in the liquid condition, and that it may be a direct result of a shortage of moisture for the enlargement of the growing kernel while in this condition. The writers have found no evidence for this assumption; and, by using the content of phosphorus as a criterion of the age of kernels, have come to the conclusion that shriveling of the kernels takes place after they have passed the liquid condition, have jelled, and should be approaching the solid stage. The water requirement of the trees during the hot months of July, August, and early September is no doubt enormous, and during this period the soil moisture supply may fall

below the wilting point of the trees.[2] In the competition for available water the leaves may perhaps draw some of their supply at the expense of the kernel. This, in addition to direct losses of water from the husk, may divert to the husk part of the water supply that normally would go to the kernel. Such action is the greater especially in districts that experience high temperature and low humidity. It may also be more extreme on trees that have many of the nuts exposed to the direct heat of the sun. A combination of all of these unfavorable factors may bring about shriveling of the kernels and may thereby reduce the value of the crop.

Experimentation

Walnut kernels collected on September 17 were placed in an oven at 65° C. After several weeks the kernel coat became light russet, but the meat remained white and there was no perceptible shrinkage. Kernels that were collected in July, just past the gel stage, however, not only shriveled badly but became dark colored when dried at 65° C. The crucial stage in the development of the kernel appears to come just before the time of solidification. If during this period the withdrawal of water is excessive, and the intake or translocation of carbohydrate material is reduced, a shriveled condition of the kernel may be expected. The intake of carbohydrate material by the kernels may be reduced by drought or by aphis infestation.

Kernels were secured from walnut trees on July 13 in Riverside, California, and again from the same trees on September 23, for observation of changes in the fresh weight, dry weight, and ash content during this important period. Table I shows the results obtained.

The kernels of the seedling and Payne walnuts obtained in July and in September show in two cases a loss in fresh weight with the increased age of the kernel, while in the case of Placentia kernels there was an increase in weight. During this period there was an enormous increase in the dry weight of all the kernels, and the ash about doubled its value. The ash as a percentage of the fresh weight increased about twice its value, except in the case of Placentia kernels, where the increase was not so great. The ash as a percentage

[2] BATCHELOR, L. D., and REED, H. S., The seasonal variation of the soil moisture in a walnut grove in relation to the hygroscopic coefficient, Calif. Agric. Exp. Sta., Tech. Paper 10. 1–31. 1923.

of the dry matter in July ranged from 6.3 to 7.1 for all the varieties, and in September the percentages lay between 1.7 and 2.5, which shows that during this period the ash increased much less than the dry matter.

When the mature Placentia walnuts were harvested from the 17-year-old trees on the irrigation plots being conducted near Irvine, California, representative samples of nuts were cracked for grading. In so doing kernels of varying degrees of shriveling were obtained, and these were classified according to the amount of shrinkage.

TABLE I

CHANGES IN FRESH WEIGHT, DRY WEIGHT, AND ASH CONTENT OF KERNELS OF THREE WALNUT VARIETIES

	PLACENTIA		PAYNE		SEEDLING	
	Date (1926)					
	6/13	9/21	6/13	9/17	6/12	9/23
Condition of kernel	Solid	Solid	Gel	Solid	Solid	Solid
Average* fresh weight per kernel	4.465 gm.	6.573 gm.	7.615 gm.	7.421 gm.	4.217 gm.	3.578 gm.
Average dry weight per kernel	0.709	4.982	0.786	5.286	0.667	3.475
Average ash per kernel	0.047	0.091	0.530	0.104	0.045	0.085
Ash as percentage of fresh weight	1.05	1.38	0.70	1.40	1.07	2.38
Ash as percentage of dry matter	6.63	1.84	6.73	1.97	6.69	2.45

* Average of from 3 to 4 analyses with from 3 to 15 kernels per analysis.

Table II shows the results of analyses made upon normal mature kernels and upon those having various degrees of shriveling.

Five kernels of each lot were used for a single analysis, and the results in table II represent the average of four such closely agreeing analyses. With an increase in the amount of shriveling there was an increase in the percentage of ash in the dry matter. This gives an erroneous view of the situation, because the fresh weight, dry weight, and amount of ash per kernel all decrease with an increase in the shriveling. The decrease in the dry matter, however, is so large as to make the slowly decreasing amount of ash appear as an increase when the ash is calculated as a percentage of the dry matter. When the constituents of the ash are calculated as a percentage of the ash there appears to be an increase in the percentage of sodium,

potassium, calcium, sulphate, and inorganic phosphate with an increase in amount of shriveling.

Walnut kernels were obtained at different times of the year from the same trees in several locations and their ash composition determined. Ten kernels were used for a single analysis, and the values given in table III represent the average of four such closely agreeing

TABLE II

EFFECT OF SHRIVELING UPON COMPOSITION OF MATURE WALNUT KERNELS

	NOT SHRIVELED	ONE-FOURTH SHRIVELED	HALF SHRIVELED	THREE-QUARTERS SHRIVELED	FULLY SHRIVELED
	Ash as percentage of dry matter				
	2.24	2.81	3.22	3.77	4.84
	Ash constituents (percentage of ash)				
Na	6.11	7.20	7.86	8.11	8.66
K	21.54	24.90	25.47	28.06	29.28
Ca	3.61	4.77	5.75	6.18	7.39
Mg	2.00	1.88	1.65	2.91	2.78
SO_4	0.83	1.68	2.07	2.52	2.66
PO_4	29.35	32.63	38.80	37.94	44.24
	Grams per kernel				
Fresh weight	4.9642	3.5034	2.9947	2.3623	1.4207
Dry weight	4.8199	3.3836	2.8801	2.2585	1.3477
Ash	0.1242	0.0951	0.0916	0.0834	0.0640

analyses. The amount of dry matter increases at a more rapid rate than does the ash, and therefore the ash as a percentage of the dry matter shows a decrease as the season advances. The percentages of sodium, potassium, calcium, and sulphate in the ash show a decrease with an increase in the age of the kernels. The percentage of magnesium shows an increase, however, as does that of the inorganic phosphate in some varieties. The amount of each constituent of the ash per kernel in general shows an increase with the increased age of the kernel.

The effect of the degree of shriveling upon the total phosphorus content of mature kernels from the 17-year-old Placentia trees at Irvine is seen in table IV. The magnesium nitrate method was used

TABLE III

COMPOSITION OF WALNUT KERNELS AT DIFFERENT STAGES OF DEVELOPMENT

	Placentia (Citrus Experiment Station, Box Springs field)			Payne (Riverside)		Seedling (Riverside)	
	7/13/26	9/21/26	10/20/26	7/13/26	9/17/26	7/12/26	9/23/26
Ash as percentage of dry matter	6.72	1.82	2.35	6.73	1.97	6.92	2.51
	Ash constituents (percentage of ash)						
Na	12.32	7.07	6.61	12.59	6.78	14.36	8.87
K	34.64	26.23	22.99	36.70	21.15	35.74	24.99
Ca	7.15	3.99	3.22	6.89	5.39	6.58	4.25
Mg	5.82	7.48	8.97	5.24	9.43	7.28	7.92
Cl	0.68	0.48	.....	.....	.....	.....	.....
SO_4	3.77	0.98	1.02	2.59	1.15	1.75	1.11
PO_4	45.88	51.54	60.38	45.94	44.37	55.65	56.17
	Grams per kernel						
Fresh weight	4.4652	6.573	7.906	7.6157	7.4216	4.7166	3.4023
Dry weight	0.7087	4.9828	5.034	0.7858	5.2862	0.6672	3.3009
Ash	0.0474	0.0909	0.1032	0.0529	0.1099	0.0445	0.0828
Na	0.0058	0.0064	0.0076	0.0066	0.0069	0.0064	0.0073
K	0.0164	0.0210	0.0250	0.0193	0.0219	0.0159	0.0206
Ca	0.0034	0.0036	0.0036	0.0036	0.0056	0.0029	0.0035
Mg	0.0027	0.0068	0.0093	0.0028	0.0098	0.0033	0.0066
Cl	0.0003	0.0005	.....	.....	.....	.....	.....
SO_4	0.0018	0.0009	0.0011	0.0016	0.0012	0.0008	0.0009
PO_4	0.0216	0.0468	0.0622	0.0239	0.0475	0.0247	0.0465

TABLE IV

EFFECT OF DEGREE OF SHRIVELING ON TOTAL PHOSPHORUS CONTENT OF FIVE KERNELS

Lot	Condition of kernels	Dry weight (gm.)	Total phosphorus (gm.)
I	Normal	20.1422	0.0387
	One-fourth shriveled	16.6659	0.0373
	One-half shriveled	14.4557	0.0378
	Three-quarters shriveled	11.2879	0.0392
II	Normal	20.9800	0.0357
	One-fourth shriveled	11.6137	0.0372
	One-half shriveled	9.6418	0.0389
	Three-quarters shriveled	8.0922	0.0390
	Fully shriveled	4.7416	0.0358
III	Normal	18.6468	.....
	One-fourth shriveled	14.2174	0.0360
	One-half shriveled	13.2421	0.0396
	Three-quarters shriveled	9.9425	0.0382

to determine the total phosphorus in five kernels of a given lot. With an increased amount of shriveling, there was a reduction in the dry weight of the kernels. It will be seen, however, that there are no important differences in the total phosphorus content of the kernels, regardless of the degree of shriveling.

Although in table II a reduction in the amount of ash accompanies an increase in the degree of shriveling of the kernel, table IV makes it clear that there is no reduction in the amount of total phosphorus.

TABLE V

TOTAL PHOSPHORUS CONTENT OF PLACENTIA WALNUT KERNELS AT DIFFERENT AGES DURING 1927

	Date (1927)					
	June 15	June 30	August 2	August 11	September 27	October 5
	Kernel contents					
	Liquid	Gel	Solid	Solid	Solid	Solid
	Grams per 5 kernels					
Fresh weight	20.019	30.046	31.309	26.168	27.610	27.530
Total P	0.0125	0.0184	0.0408	0.0467	0.0678	0.0830
Total P calculated as PO_4	0.0384	0.0563	0.1254	0.1430	0.2077	0.2543

The total phosphorus content of shriveled kernels is about the same as that of normal kernels, and hence the shriveling must take place after the kernel has received its full complement of total phosphorus, or else phorphorus absorption may still go on uninhibited by the shriveling process. It seems of interest, therefore, to make a study of the changes in the total phosphorus content of walnut kernels of different ages.

Placentia walnut trees were selected in the field at the Citrus Experiment Station, and at intervals as shown in table V samples of nuts were taken for analysis. On June 15 the kernel contents were still in the liquid condition. By careful manipulation the kernels were removed from inside the husks without losing any of their contents. Fig. 2 shows the appearance of the kernels at this time, with

some cut open to show the interior. By June 23 the tip and suture of the shells were becoming woody, and by June 30 the entire shell was woody. By July 7 the kernels were still liquid in the center, but firmly jelled along the inner surfaces of the kernel. At the time of the last sampling, the nuts were mature and ready to fall from the husks. A large number of nuts still in the husks were left after the determinations of August 2. These were left standing on the table, and on August 10, although some drying had occurred, the total phosphorus had not changed from that given for August 2. From table V it is seen that there is a progressive increase in the total phosphorus of walnut kernels as they increase in age. A comparison of tables IV and V shows a wide difference in total phosphorus between walnut kernels from the Riverside district and ones from the Irvine district.

Summary

1. The shriveling of walnut kernels takes place after they have passed the liquid condition, have jelled, and are approaching the solid stage.

2. The fresh weight, dry weight, and amount of ash per kernel all decrease with an increase in the shriveling. The ash as a percentage of the dry matter increases with an increase in the amount of shriveling.

3. The amount of each inorganic constituent of the ash per kernel in general shows an increase with the increased age of the kernel.

4. The degree of shriveling has no effect on the total phosphorus content of mature walnut kernels obtained from trees growing on a given soil type.

5. There is a progressive increase in the total phosphorus of walnut kernels as they increase in age.

6. Normal mature walnut kernels obtained from trees growing on widely differing soil types show differences in their total phosphorus content.

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BRIEFER ARTICLES

TECHNICAL CONTRIBUTIONS

I. IMPROVED METHOD OF SOFTENING HARD TISSUES

In various contributions, methods have been described for studying microscopically the organization of hard vegetable materials such as coal, carbonized fossil plants, dense tropical woods, etc. In the case of the more refractory of these substances, the prolonged treatment necessary for adequate softening has proved a disadvantage; and the tissues themselves in some instances suffer modification from too lengthy treatment. As a consequence, experiments have been carried on during the past two years for the purpose of shortening and at the same time improving the technical manipulation of hard vegetable objects. These experiments have now reached a successful conclusion, and in view of the considerable interest which they have aroused they are published herewith.

The fundamental condition in the newer technique of softening vegetable tissues is the use of the somewhat high temperature employed in the vulcanization of rubber. An ordinary dental vulcanizer has proved to be very satisfactory, especially as the small ones suitable for this purpose are not very expensive. Ordinarily they are equipped with a thermoregulator which controls the temperature at 320° F. At first the material was raised to the vulcanizing temperature in water, as boiling water is ordinarily employed in preparing woods for sectioning. This procedure answered fairly well in many instances, but unless care was taken there often resulted an extreme degree of hydration which made the tissues friable and difficult to section. It was considered probable that the use of alcohol of various grades would overcome this difficulty. In 95 per cent alcohol very little hydration or disintegration occurs and the period for softening is somewhat prolonged. For example, a piece of white or red oak will require three or four hours or even more in the thermostat for proper softening. If 70 or 50 per cent alcohol is used the length of time required is shorter, but the danger of deterioration is greater. If pure water is used with oak half an hour is quite sufficient, but the result is not nearly so good as that obtained by exposing the same material in 95 per cent alcohol for four hours. After treatment in the vulcanizer a very brief action of hydrofluoric acid is sufficient. Even in the case of the

hardest materials, two or three days' exposure to hydrofluoric acid of one-half or one-third the commercial strength is all that is necessary.

One great advantage of the new method is that it is possible to treat with it tissues which are partly hard and partly soft; for example, the branches of oak and hickory are more or less difficult to soften satisfactorily by the older method. When vulcanized in 95 per cent alcohol they are quickly mastered, and are much more homogeneous than they are as the result of prolonged treatment in hydrofluoric acid alone. The time of exposure of a three- or four-year old branch of oak naturally is much shorter than for a piece of dry oak wood. An hour generally suffices, in contrast to the four or five hours required for the wood.

The technique of preparing the material for vulcanization in alcohol is of considerable importance, although easily and inexpensively practiced. Pieces of "iron-size" brass pipe 0.75–1 inch in diameter are cut to fit in the vulcanizer. These short lengths of pipe are threaded at each end for a brass cap of appropriate size. One end is capped and the cap is made vapor-tight by having lead solder "sweated" into the thread. The cap on the other end has inserted within it a plate of lead against which the smooth end of the pipe fits. The lead gasket, however, does not serve alone to prevent the loss of alcohol at high temperatures. It has been found desirable to insert a disk of cork, fiber, or common cardboard between the lead gasket and the end of the pipe, thus securing a perfectly tight joint even with strong alcohol. The brass pipe is fastened in a pipe-vise, and after the material has been introduced in alcohol the cap is firmly screwed on with a Stillson wrench. Obviously a number of samples can be treated in the same or different tubes in one operation, with a corresponding economy of time. After vulcanizing the material the vulcanizer is allowed to cool slowly, or if haste is necessary the steam is allowed to escape and the hot brass tubes are cooled under a faucet. When the material is removed from the tube it needs only a comparatively short sojourn in hydrofluoric acid. Admirable results have been obtained without imbedding in the case of such refractory materials as peach stones, coconut shells, and the shells of a great number of nuts. Equally good results have been secured in the case of such woods as oak and lignum vitae. After these various hard objects have been vulcanized they are treated for a few days in a mixture of one to two parts of water to one of strong hydrofluoric acid. After being well washed in water they are run up in alcohol and finally transferred to a mixture of equal parts of alcohol and glycerin, as in the earlier methods.

There is no comparison between the results to be obtained in this way

and those secured by the older technique. For example, it is possible to cut continuously transverse sections of coconut shells and hard woods only 2 or 3 μ thick. The wealth of detail shown by these sections which are entirely without scratch or dragging on the part of the knife is almost beyond belief. Every feature of pitting stands out with diagrammatic clearness, and the most difficult details can readily be studied with the use of an oil immersion. The treatment here described yields such satisfactory results that it seems to mark an important step forward in the difficult field of the study of hard tissues of plants.

II. MASS METHOD OF IMBEDDING

In the practice of microtomy, occasions often arise where it is a great advantage to be able to cut a large number of small objects at the same time, such as the carpels of the rose, or the small flowers of the sedges, grasses, or Compositae. Obviously, if the carpels of the rose are sectioned as they lie in the young fruit, or the flowers of the groups mentioned are cut on the microtome in heads, comparatively few of them will reveal the median section which is of such value in arriving at conclusions in any given case. This situation presents itself in its most difficult form in the study of the reduction division, particularly in ovules and very small anthers. In the case of ovules there are comparatively few mother cells undergoing division in a given quantity of material, and the same statement applies to the small anthers of the minute flowers of many groups of plants. In such instances it is obviously advantageous to cut great quantities of material at once and in the desired plane of section. The mass method here described has been developed to meet these difficulties.

The material, however preserved (the method varying of course in different cases), is run up into strong alcohol, and then transferred to a mixture of equal parts of alcohol and glycerin in which it should stay at least overnight. When the glycerin-alcohol has passed into the material, the objects are removed from it and handled under the low power of the dissecting microscope. Let us suppose that the ovary of a rose is to be studied in order to discover the behavior of the chromosomes in the heterotypic divisions of the embyro sac mother cell. Before transferring to glycerin-alcohol the top of the flower has been cut off below the level of the stigmas, and a similar section at the time of preservation has laid open the base of the receptacle. The young flower is placed on the stage of the dissecting microscope, and with needles or fine forceps has the base of the calyx surrounding the carpels removed. The bunch of carpels is then transferred to a piece of heavy paraffin paper in a small drop of

equal parts glycerin and alcohol. The carpels are separated from one another by needles or forceps and laid out in a row on the piece of paper. Obviously, several flowers may be advantageously manipulated at once. After the carpels are laid out properly, the strip of paper is put in a warm place to encourage evaporation of the alcohol.

Previously a supply of glycerin jelly is prepared by using pulverized gelatin, purchasable at any grocery. The gelatin is put in warm water in the proportion of one ounce of gelatin to six ounces of water. After the gelatin is thoroughly dissolved, which happens very quickly if it is pulverized, an equal volume of glycerin is added. The jelly so produced resembles closely that ordinarily employed for mounting certain microscopic objects. It is, however, unnecessary to filter or clarify it for the present purpose. Further, unless a very large stock is made up it is unnecessary to use an antiseptic, as the glycerin serves this purpose sufficiently.

Cardboard, which need not be of a very high grade, is cut up into strips of a convenient width, depending on the particular material used. Eighteen mm. is a generally useful dimension, but sometimes broader strips are required. Some of the glycerin jelly is heated to the melting point and spread with a glass rod or the limbs of a forceps evenly over a small strip of cardboard. The objects which have been arranged on paraffin paper in any desired order, having in the meantime lost their excess of alcohol, are inverted and applied to the melted glycerin jelly, the paraffin paper naturally being uppermost in this operation. After the paraffin paper has been placed over the glycerin jelly, an ordinary microscope slide is laid on the top of the paper and on the slide is placed a small weight which flattens out all the rose carpels parallel to the surface of the cardboard. When the jelly has set (from half an hour to two hours is usually long enough), the strips of cardboard with their adhering paraffin paper are put in 90 per cent alcohol, which brings about the hardening of the jelly. Sometime afterward, it may be next morning, the cardboard strips are placed in a watch glass of alcohol. The paraffin paper has now become translucent, and it is possible at this stage to proceed to the further preparation of the material. This is effected by pricking with a fine (no. 12) needle inserted in a cork. The pricking should be somewhat uniform, but care should be taken to avoid perforating parts likely to be injured by that operation. The paraffin paper may now be pulled off. This method has been arranged especially for nitrocellulose infiltration. It is possible by means of a comparatively few sections through the median plane of a large number of carpels to secure a large amount of important information.

The gelatin used in this method, if employed too abundantly, constitutes a deeply staining matrix surrounding the objects. By using a thin layer of gelatin it is possible to bring about the adhesion of the objects without any of these disadvantages.

The objects prepared by this method stain very well and supply most useful preparations. By the employment of this method it is possible to use material for class purposes which without this procedure could not possibly be so utilized. Doubtless this method could also be employed with paraffin, but it has proved its great utility in connection with the imbedding of material in nitrocellulose.

III. SLIDING MICROTOME FOR CUTTING HARD TISSUES

(WITH ONE FIGURE)

An outstanding necessity for American biologists has been a microtome on the sliding principle, which would combine extreme accuracy with the possibility of securing thin sections, particularly of hard tissues. A microtome, fulfilling these requirements to a very large degree, has been devised by Professor R. B. THOMSON of the University of Toronto, and has been so suitable for its purpose that it has attained a wide although somewhat limited sale. The price of this microtome, in the neighborhood of $500.00 in Toronto where it was manufactured, has put it beyond the reach of most workers in other countries, particularly since the war and the consequent prevalence of high tariffs.

A microtome of this type has been so much a desideratum for American workers in recent years, particularly as a result of the increased interests in anatomy and the greater use of nitrocellulose as an imbedding medium for all purposes, that the writer has considered the construction of a modification of this microtome. The cost of the Thomson instrument is due very largely to the somewhat expensive appliances which have been introduced into its structure, chief of which is the apparatus for raising, lowering, and tilting the knife. This feature of the Thomson instrument has been admirably designed, but is too elaborate and expensive for general use. The writer has taken up the subject of modification of the Thomson microtome with several firms of instrument makers, with the result that the Bausch and Lomb Optical Company three years ago undertook to manufacture and put on the market a modified Thomson microtome.

The new microtome (fig. 1) is provided with a micrometer screw of the clicking type. This differs from the one supplied with the first instrument and has certain practical advantages over it. The micrometer

screw on the original instrument is modeled after a type now commonly used in which the movement of the screw is controlled by a lever and ratchet. The lever in this type of appliance, if used with the vigor necessary to overcome the inertia of the rather heavy block which supports the object, stubs against the rod which controls the thickness of the section. This is injurious to the micrometer apparatus, and there is great difficulty in securing the particularly thin sections which are desirable, not only in difficult investigations, but also for general use in the classroom. The older type of micrometer screw, in which a knife-edge engages in a transverse groove of a disk attached to the screw, is of greater practical advantage, since it permits much smarter and more vigorous action, which is necessary to overcome the inertia of the object-holder, particularly in the case of very thin sections. Further, the operation does not endanger any part of the screw itself. The only fault which can develop is over-shooting the required thickness, a defect which is quickly remedied as the microtome becomes easier running by use and the operator has acquired skill and experience.

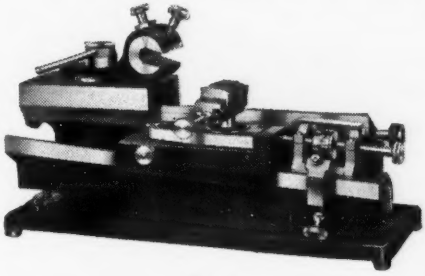

FIG. 1

The instrument is constructed of nickeled steel, and in it the three point bearings are inverted from the position ordinarily found in sliding microtomes of European manufacture. This inversion makes for greater rigidity and solidity in operation, and appears to constitute a decided feature of advantage in the Thomson instrument as well as in its modified form here described. The object-holder has a very efficient universal joint which is so large that great rigidity results. The object clamp is a very practical type, and is provided with three different insets for cutting round objects of various sizes. This feature is highly advantageous. The object-holder is pushed up an inclined plane which has a gradient of one in twenty, as in the well known Jung-Thoma microtome, which has supplied the needs of American workers for many years. The knife-holder, as is usually the case, runs on horizontal ways. The knife is held by a tilting knife-holder which can be oriented at any angle in the vertical and horizontal planes. The raising of the knife for long objects is attained

in the new instrument by the efficient and inexpensive device of plane-parallel plates. A number of these are supplied, and permit a considerable range of height.

The new instrument has been thoroughly tested in the writer's laboratory. It is accordingly probable that it will not develop any important defects when used for the purposes for which it was designed, namely, the sectioning of hard tissues and of objects imbedded in nitrocellulose. In use it has proved equally advantageous for animal and vegetable material, and it is confidently recommended to American biologists as an instrument which will give the maximum of performance at the lowest consistent price.

IV. IMPROVEMENTS IN USE OF NITROCELLULOSE FOR IMBEDDING

(WITH ONE FIGURE)

Imbedding in nitrocellulose has become more and more important in the technique of both plants and animals in recent years. It is now possible to cut sections as thin as those which may be prepared by the paraffin method in the most favorable cases. Not only is nitrocellulose now applicable to the more delicate tissues of plants, but it is still the method par excellence for hard tissues and for structures combining thick- and thin-walled cells. Under certain conditions it may produce shrinkage, in spite of its well deserved reputation for causing less disturbance in this respect than does paraffin. The general technique which has proved of value in the writer's laboratory is adequately described by CHAMBERLAIN,[1] or at greater length by the writer.[2]

It has been found desirable, in connection with cytological investigations on the more delicate parts of plants, and on more delicate organisms than have previously occupied attention, to develop improvements in the technique of imbedding in nitrocellulose. These improvements offer advantages in connection with staining, and furnish distinctly clearer pictures than can be obtained with the paraffin method.

It will be convenient to consider first the preparation of very delicate structures for the purpose of imbedding. Perhaps the most delicate and difficult objects in this connection are the green and brown algae, as, for example, *Spirogyra* and *Fucus*. The classic and apparently the most advantageous preservative in these cases is chromic acid, or rather chrom-acetic varying in strength from one to a fraction of one per cent, depend-

[1] CHAMBERLAIN, C. J., Methods in plant histology. 4th ed. 1924 (pp. 127–129).

[2] JEFFREY, E. C., Anatomy of woody plants. 1917 (pp. 449–451).

ing on circumstances. The material of *Spirogyra* is dropped without preparation into the preservative. In the case of *Fucus* it is desirable to cut off the ends of the so-called receptacles, so that the chromic acid may penetrate readily. In both instances it is advisable to use an air pump (to be subsequently described) in the field, for the purpose of removing the gases and promoting penetration of the preservative. After remaining for one or two days in the preserving fluid, the material is washed with water to remove all excess of chromic acid. If the material is not to be used at once, it may be kept indefinitely in water to which 5 per cent phenol has been added. In case of the brown seaweeds it is desirable to demineralize before imbedding, as there is some mineralization of the thallus near the surface which interferes with the preparation of thin sections for cytological study. The removal of mineral matter is effected by weak hydrofluoric acid (not stronger than 5–10 per cent). Washing removes the acid, and the material is now exposed to the action of 10–15 per cent glycerin in water. The glycerin is concentrated by the slow evaporation of the water, which is facilitated by a very slight degree of warmth. This procedure is commonly adopted for the purpose of making total preparations of algae, but is here utilized for making sections free from shrinkage in the case of even the most delicate objects.

After the glycerin has become concentrated by slow evaporation of the water, the material is transferred to strong alcohol (90–95 per cent). If the objects are massive they do not require any special handling, but in the case of *Spirogyra* and other minute or filamentous algae it is necessary to attach a mass of the material to a piece of cardboard by means of the mass method previously described. To effect this, the material of *Spirogyra*, *Chara*, or *Nitella*, etc., is changed from strong alcohol to equal parts of alcohol and glycerin. From this it is transferred to blotting or filter paper, to allow some of the alcohol to evaporate. A mass is then lifted to a piece of cardboard coated with a thin layer of glycerin jelly, and then gently weighted down after having been covered with a piece of heavy paraffin paper and a glass microscopic slide. This is preliminary to imbedding. In the case of *Fucus*, *Laminaria*, etc., it is often desirable to prick the material to some extent with a fine needle, to permit subsequent penetration of nitrocellulose. Whether the algae are small or large (the same treatment applies to aquatic and other fungi), they are now transferred to strong alcohol and the air removed with a powerful air pump. They are then dehydrated in the usual manner by successive changes of absolute alcohol, three in number. The material now passes in 12-hour periods through nitrocellulose in 2, 4, 6, 8, and 10 per cent

solutions, which are heated in a carefully wired bottle in the bath. The temperature of the bath may be 50°–65° C. and even hotter. The hot nitrocellulose penetrates much more thoroughly and much more quickly than when used cold, and very thin sections can readily be secured as a consequence. Instead of passing the material through successive higher grades of nitrocellulose, as is the case with less delicate material, the thickening process starts with the 10 per cent solution.

Fig. 2 will make clear how this is brought about. A piece of copper or zinc is cut into strips about an inch wide with a pair of tinsmith's "snips." These strips are cut crosswise at intervals of two inches and then one end is sharpened to an arrow-shaped point. The strip of metal is then folded lengthwise through the middle over a piece of hard wood to

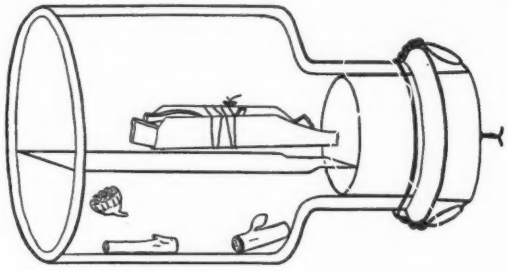

FIG. 2

an angle of 90°. One or two turns of soft brass wire are wrapped round the resulting trough somewhere near the middle. Under this wire are pushed strips of parlodion or photoxylin until wedging occurs. The material in 10 per cent nitrocellulose is now ready for the thickening process. This is effected by driving the pointed end of the metal trough, with the concavity upward, into the inner end of the cork. This should be done so that the lower side of the trough does not more than touch the surface of the nitrocellulose when the bottle is laid in a horizontal position. The bottle is then securely wired and put into the warm bath. At the end of 24 hours it is cooled off and the cork withdrawn. A second trough filled with nitrocellulose is substituted for the first, and after a sojourn of 24 hours the process is repeated.

This method has the advantage of bringing about a very gradual concentration of the nitrocellulose which is superior to the ordinary evaporation method. In the method by evaporation the ether passes off from the solution more readily than the alcohol, and a more or less cartilaginous

mass results which neither supports nor penetrates the object thoroughly. By the method described, alcohol and ether are equally absorbed by the suspended nitrocellulose. There is consequently no precipitation due to excessive evaporation of one of the solvents; and shrinkage, such as results from the direct placing of chips of nitrocellulose in the dissolved medium, is also avoided. It is obvious that it is possible by this method to make the thickening extremely gradual and also progressive. This makes it possible to imbed the most delicate algae and fungi without shrinkage. After hardening the nitrocellulose in the usual manner, sections may be cut readily 5 μ or even thinner. This procedure has proved of great value in manipulating saprophytes, fungi, etc., which on account of their poorly developed air spaces are apt to shrink with the ordinary method. For example, the ovules, ovaries, and fungus covered roots of our two species of *Monotropa* can readily be prepared for sectioning in this fashion. The same statement holds true for the delicate mycorhizas of the orchids, which constitute an exceedingly difficult object with the usual technique.

It may appear that the process of imbedding here described is too elaborate for ordinary use. The main labor, however, is in getting the material into 10 per cent nitrocellulose. After that stage has been reached the thickening of the imbedding medium is practically automatic. The sections which result are very clear, and serve admirably for photomicrographic purposes. They may be stained in almost any desired manner, provided the staining is effected slowly by weak solutions of the dyes. Haidenhain's haematoxylin works equally well with the various anilines.

V. AIR PUMP FOR THE FIELD

(WITH ONE FIGURE)

It has been realized more and more in recent years that the exhaustion of air from vegetable tissues is a prime necessity from the standpoint of good preservation. When preservation is carried on in the laboratory the electric air pump, which has become a necessary adjunct of modern biological laboratories, is available for this purpose. Since, however, a very large part and a vitally important part of the preservation of material must be effected in the field, a suitable air pump for use under field conditions is highly desirable. The automobile pump is the inexpensive and entirely satisfactory basis for such an instrument. The hand pump for the automobile is constructed to force air into the inner tube of a tire. It is possible with a few slight changes to make it function efficiently as a producer of negative pressure. The left-hand items of fig. 3 show how this is effected. All that is necessary is to reverse the leather

plunger, and in so doing make sure that it is held firmly in place in its new position. In some pumps it will be necessary to put a washer on the plunger rod, just above the leather piston, as otherwise the piston will not fit tightly in place. When the nut which holds the piston is screwed up it is well to rivet the end of the rod, so that the nut will not come off and thus cause annoyance in the field. Care should be taken also to have a spiral spring between the top of the plunger and the cap of the pump, to take up the shock of the upward stroke and thus prevent the cap from being torn off. Usually there is a ball valve at the base of the hose attached to the pump. By unscrewing the nipple to which the hose is attached the ball can be readily extracted. This is a necessary operation because the ball valve responds to positive pressure and gets in the way of negative action.

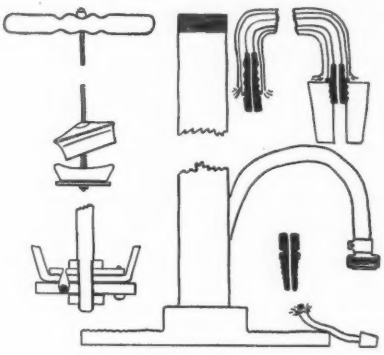

FIG. 3

The hose from the pump has attached to it the valve of the inner tube of an automobile tire. This is wired into a short length of rubber hose which at the other end has another valve from a disused inner tube. The rubber plunger or valve inside is removed from one or both of the valves. In certain instances it is an advantage to keep it in the valve farthest away from the attachment. This valve is inserted into a rubber stopper which fits the bottles used for preserving. The details of these structures are represented on the right of fig. 3.

To use the pump, the rubber stopper is inserted into the bottle containing the preserving solution and the material to be preserved. The plunger is drawn smartly upward but the stroke stops short of the air vent in the side of the cylinder just below the cap. In this position a high vacuum is instantly produced, which causes the air to gush out of the material. As soon as the flow of air has diminished, the plunger is drawn to the top and the vent allows the air pressure to pass off. The plunger is then forced down slowly and drawn back with another sharp stroke. Four or five strokes are sufficient to cause most objects to saturate and sink in the preserving fluid. When an automobile pump is used, a valve inside is not necessary in the tube inserted in the rubber stopper,

as the space in the cylinder of the pump is relatively large compared with the bottle to be exhausted. If the automobile pump is replaced by a bicycle pump, as has been suggested by Professor R. H. WETMORE, a valve is essential on account of the short stroke and the narrow diameter of the pump. Practically the valve has been found unsatisfactory, however, on account of the frequent attention which it requires, and its very short life resulting from the corrosive vapors to which it is exposed. The writer for that reason does not use a valve at all.

The piston of the pump is most suitable for the purpose, if provided with a ball valve as shown on the left of the illustration. The advantage of the ball valve is that the piston in its downward stroke allows air to pass through readily, and does not force the cork out of the bottle which is being exhausted. Care should be taken to clean and grease the leather of the piston frequently, on account of the character of the preservative fluids used for the fixation of the tissues.

This pump has been found useful, not only for vegetable tissues but for animals as well, as in all cases it favors the rapid penetration of the preserving fluid. A further advantage is the fact that a smaller amount of preservative can be safely used and larger pieces of material than where no pump is used. This appliance has now been in use in the writer's laboratory for nearly a decade, and the present account is supplied as an easy way of answering numerous inquiries on this subject.—E. C. JEFFREY, *Harvard University.*

CURRENT LITERATURE

BOOK REVIEWS

Morphology of fungi

During the past 20 years, botanical literature has been accumulating at such a rate that it has become increasingly difficult for investigators, especially those of the younger generation, to make sure that they are not overlooking work already done. *Botanical Abstracts*, and now *Biological Abstracts*, help to minimize the danger. Fortunately, it has been recognized that the danger exists, and useful books are appearing which put the student into contact with the most important literature in a given field and lessen the drudgery of reference library work. Some notable examples of this type of book are *Cytologie der Blüthenpflanzen*, by SCHÜRHOFF; *Anatomie der Angiospermen Samen*, by NETOLITZKY; and in our own country, the works on cytology by WILSON and SHARP. In all these books a clear presentation of the subject, with very extensive bibliographies, greatly facilitate research.

A recent work of this character, which will be of great value to all who are studying fungi, is DODGE'S[1] translation of GAÜMANN'S *Vergleichende Morphologie der Pilze*.

The book is more than a translation, for the new literature which has appeared since 1925 has been incorporated, and in some places the recent results have necessitated a rewriting of the group concerned. Some of the more important of these changes are seen in the treatment of the Basidiobolae, the Elaphomycetaceae, the stromatic Sphaeriales, the Laboulbeniales, the Thelephoraceae, the Hydnaceae, the Gasteromycetes, and the Uredinales. An extensive rearrangement of orders was made with GAÜMANN'S approval. Unfortunately, Dr. GAÜMANN'S illness made it impossible for him to collaborate in the work.

The chapter headings indicate the arrangement of groups and suggest something of the mode of treatment. They include a discussion of the Thallus, Reproductive organs, Sexual organs and sexuality, Archimycetes, Phycomycetes, Chytridiales, Oomycetes, Zygomycetes, Ascomycetes, Hemiascomycetes, Taphrinales, Euascomycetes, Perisporiales, Myriangiales, Hypocreales, Sphaeriales, Dothideales, Hysteriales, Hemisphaeriales, Phacidiales, Pezizales, Tuberales, Laboulbeniales, Basidiomycetes, Polyporales, Agaricales, Gasteromycetes, Tremellales, Cantharellales, Dacryomycetales, Auriculariales, Uredinales, Ustilaginales, and Fungi Imperfecti.

[1] DODGE, C. W., Comparative morphology of fungi. 8vo. pp. xix+701. *figs. 406* and *43 diagrams*. New York: McGraw-Hill Book Co. 1928.

The book is written from the standpoint of morphology, with cytological and phylogenetic features everywhere in evidence, the opening paragraphs introducing the x and $2x$ features of alternation of generations. The statement that when the x and $2x$ phases follow each other as two morphologically different generations we have alternation of generations is true; but the reviewer would contend that there is alternation of generations wherever there is sexuality, whether the two generations look alike or different. That the x generation is called the gametophyte and the $2x$ generation the sporophyte is true, and it happens to be correct for plants above the Thallophytes, but is unfortunate for the algae and fungi, where the x generation so often produces both gametes and spores. We must also take exception to the statement that the $2x$ phase is "reduced to a zygote." More probably it has not been reduced; but rather the reduction division, bringing back the x phase, has simply taken place early, before a more extensive $2x$ phase could be built up. This short $2x$ condition should be expected in the evolution of alternation of generations at the time when reproduction by gametes began to emerge from the zoospore condition.

The fact, however, that alternation of generations is recognized and emphasized, gives the book a freshness not found in works where the morphology is limited to a study of disconnected life histories, with the control of diseases as the only object.

The treatment of the Laboulbeniales and Uredinales is particularly full, and is rewritten and revised rather than translated. Similar but less extensive changes are found throughout the book.

The illustrations are numerous and well chosen. As should be expected in a work of this scope, very few are original. Some of the figures are taken from papers which appeared after GAÜMANN'S book was published.

To mention individual details would require more space than can be given to a review. The whole treatment is from the standpoint of comparative morphology, with cytology and phylogeny prominent. The clear presentation, with more than a thousand titles in the bibliography, and an effective index of 30 pages, will greatly facilitate research by putting students into contact with the literature, and systematizing the reference library work necessary in any investigation. Morphologists, pathologists, and mycologists will find this book very useful, even if they already have the German edition.—C. J. CHAMBERLAIN.

Life of plants

An attractive book on the life of plants has appeared by Sir FREDERICK KEEBLE[2] of the University of Oxford. It is an exceptionally entertaining and instructive work, intended for the lay reader and for those who are just beginning the serious study of plant life. Likening the development of science to the growth of a tree, KEEBLE attempts to show that science is much more than a mere body of doctrine, that it is, indeed, an illumination of life. Admitting the

[2] KEEBLE, F., Life of plants. 8vo. pp. xii+256. New York: Oxford University Press, American Branch. 1926. $1.75.

difficulties of presenting science in this rôle, he modestly expresses the opinion that he has failed to measure up to the task. Nevertheless, the reader will find the work an unusually illuminating introduction to the world of plant life. Botany would gain much in appreciation of its service to humanity if the knowledge to be obtained from this book were to become the possession of people generally.

This interpretation of plant life is presented in nine chapters, the first of which deals with the cosmic significance of plants, the part they play in nature, and the origin of the land flora. In the second chapter the chlorophyll-bearing and non-chlorophyllous plants are contrasted, and wheat is described as an example of useful seed plants. The third and fourth chapters consider the food-manufacturing activities of the higher plants, including the utilization of nitrogen, and the way in which radiant energy of sunlight becomes the potential energy of the foods stored in the plant body. The fifth and sixth chapters present the problems of mobilization of food materials, diffusion, osmotic transfer, the conducting system, enzymes concerned in mobilization, and the final use of foods for constructive material and vital energy.

The final chapters consider the environment of land plants, variation and heredity, and the plant commonwealth. The last chapter centers attention upon one of the most difficult problems of life, correlation of life activities, the integration and coordination of the processes upon which orderly development and the fulfilment of the purposes of life depend. This he conceives as dependent mainly upon chemical messengers, hormones, set free by the stimulative action of environmental conditions on the sensitive protoplasmic material of the cells.

The story is told in pleasing manner, and the book should prove of value as collateral reading for students who are gaining their first serious knowledge of plant life and plant processes. The reviewer considers that KEEBLE has given us an excellent elementary illumination of the life of plants as active living beings.—C. A. SHULL.

Principles of plant physiology

The greatest need with reference to textbooks in botany at present is for a good sound text in plant physiology. Few attempts have been made in recent years to meet this need, and those have been mostly abortive efforts. The latest work along this line is a text by RABER.[3] The book contains thirty-one chapters, the titles of which will not be given. The six sections, however, are: Introduction; Nutrition; Growth and movement; Reproduction and heredity; Death; and Vitalism and mechanism.

The introduction classifies the sciences, and considers the cell as the unit of living structures. The colloidal state is considered in connection with the properties of protoplasm. The section on nutrition is presented in twenty-three chapters, and falls into three divisions: Constructive metabolism; Absorption and movement of materials; Destructive metabolism.

[3] RABER, O., Principles of plant physiology. 8vo. pp. xvi+377. Macmillan. 1928.

Certain features of the arrangement seem illogical to the reviewer. For instance, the problems of absorption and movement of materials, gases, liquids, salts, etc., and the problems of transpiration and ascent of sap are more easily understood than the problems of photosynthesis, chemosynthesis, and nitrogen metabolism. They should precede the latter subjects for an easier approach to the problems of plant life. In destructive metabolism, anaerobic respiration is considered after aerobic, even though in actual life probably all respiration has an anaerobic start. The logical arrangement is to treat the subject of respiration as a whole, and start at the beginning of the process.

In some chapters the text somewhat overshoots the mark for an elementary presentation, but in the main it could be mastered by beginners. The chief criticism to be made is that there are many errors running through the text which students will not be able to detect because of lack of experience and judgment. It is not possible to go into details with very many of these errors, but a few cases will be cited. On page 50 the author indicates that BALY'S earlier work on photosynthesis rests upon careful experimental evidence. The facts are that BALY'S theory is highly hypothetical, not based upon experimental results, and not in agreement with WILLSTATTER'S published work. Again, on page 107 the author indicates that the salts in plants are maintained in constant ratios, similar to the condition of the salts in the blood of animals, and that the ratios are related to the Cambrian Sea out of which life is supposed to have arisen. This idea certainly does not receive any support from the known facts of salt distribution in the sap of plants.

On page 114 it is stated that d-xylose is optically inactive, although ARMSTRONG and others give the rotation for the α-form as $+92°$; and on page 112 an erroneous interpretation of the prefixes d- and l- for the optically active sugars is given. Take fructose, for example, levorotatory whether in the α or β form, and levorotatory at equilibrium of the two forms in mixture, yet it is a d-sugar. On page 153 the impression is given that emulsin is a single enzyme, but, like diastase and other similar preparations, it represents a group of enzymes of which amygdalase and prunase are the principal catalysts. On the following page, amygdalin is made "the essential material in oil of bitter almonds"; but oil of bitter almonds usually signifies benzaldehyde, which is a constituent of amygdalin.

On page 166 it is not made clear that litmus has no relation to the anthocyanin pigments, along with which it is mentioned. The student would get the idea that litmus belongs among the anthocyanins. On page 279 the "grand period of growth" is interpreted as the central part of the growth period, when growth is most rapid, whereas the term is used for the total growth period, from the very beginning of enlargement until growth ceases. On page 293 ethylene is credited with ripening lemons, although its action is merely to hasten chlorophyll decomposition in lemons which are internally ripe. Many other instances might be cited, but these cases are sufficient to indicate the kind of errors which occur.

While the number of these slips is disappointing, one finds that many of the

chapters are otherwise well written; some are fair in quality, and about half a dozen are rather poor. It is greatly to RABER's credit that he has attempted to write a text for the use of plant physiologists, a task which has been slighted by those well fitted by training, experience, and research to perform. A good text cannot appear unless those capable of writing one are willing to sacrifice time and other forms of scientific activity in order to produce it. Such a text should come if possible from a master teacher who also possesses ability as an investigator, and who is capable of exercising discriminating judgment as to the material which should be included in such a text.—C. A. SHULL.

First course in botany

Another elementary text has recently been prepared by POOL and EVANS.[4] The first chapters deal with customary topics: cells, roots, leaves, stems, the flower, and the seed. The emphasis is placed upon the work of these parts of the plant, with only sufficient discussion of structure to afford a basis of understanding. Then follows a chapter on the plant and its environment, with another on weeds as application of the adaptation of the organism to its environment. Chapters x to xv, 133 pages, deal with classification and with type plants. The closing chapters are on forestry, plant breeding, and the diseases of plants.

The language of the book is simple and the authors have been conservative in the introduction of scientific terminology. At the close of each chapter there are a summary and a series of questions and problems. These latter are intended to test the pupil's ability to apply the principles treated, rather than merely to recall the information acquired.

The book may be commended as an excellent text for secondary school botany. It has been written with the editorial cooperation of Dr. OTIS W. CALDWELL, and is evidently intended to bring up to date the earlier books of which he was an author.—E. R. DOWNING.

Useful plants

Aside from the usual products of farm and garden, it is surprising how few useful plants are known to the general public. To furnish means of a more extensive acquaintance, CLUTE[5] has issued a little book, written in non-technical language, describing many plants used as food, condiments, beverages, drugs, dyes, textiles, construction, and decoration. The book should be of special interest to the layman, and even the professional botanist may learn something from its pages.

In general the common names of the plants are used in the text, but there is also a list giving the botanical names and the family to which the plant belongs. The book should serve to emphasize the economic importance of all branches of plant science.—G. D. FULLER.

[4] POOL, R. J., and EVANS, A. W., First course in botany. 8vo. pp. ix+414. *figs. 219.* Ginn & Co. 1928.

[5] CLUTE, W. N., The useful plants of the world. 8vo. pp. 86. Joliet, Illinois: Publ. by W. N. Clute & Co. 1928.

NOTES FOR STUDENTS

Taxonomic notes.—TRELEASE[6] has published a new genus (*Trianaeopiper*) of Piperaceae, which includes 4 species segregated from *Piper*. The author states that these are "the only other species with axillary spikes which remain in the genus *Piper*." In range they are confined to the Colombian Mountains.

BROWN[7] has described a new genus (*Cerochlamys*) of Mesembryanthemeae from South Africa. The name refers to the waxy film that covers the leaves.

In continuing his account of African plants, GOSSWEILER[8] has described a new genus (*Dalbergiella*) of Leguminosae, a shrubby climber closely related to *Dalbergia*.

PITTIER,[9] in continuation of his investigation of the Bignoniaceae of Venezuela, has described 9 new species of *Arrabidaea*, almost doubling the number of species known in that country.

FERNALD[10] has described a new genus (*Geocaulon*) of Santalaceae, to include what has been known as *Comandra livida*. It creeps in moss or damp humus, and occurs from Labrador to Alaska and southward to northern New England and British Columbia.

ROBINSON,[11] in recording material preliminary to his publication of the Eupatorieae, has described as new genera *Mexianthus* (Mexico) and *Trychinolepis* (Peru); and also 4 new species of *Stevia* from Mexico and 12 new species of *Eupatorium* and 6 new species of *Mikania* from various South American countries.

JOHNSON,[12] in continuation of his investigation of the Boraginaceae, has published an account of the South American species of *Heliotropium*. The genus contains 73 species, 13 of which are described as new. He has also published[13] 19 new species from various South American countries in 17 genera, one of which, belonging to the Iridaceae, is new (*Mastigostyla*).

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[6] TRELEASE, W., *Trianaeopiper*, a new genus of Piperaceae. Proc. Amer. Phil. Soc. **67**:47–50. 1928.

[7] BROWN, N. E., *Cerochlamys*, a new genus of Mesembryanthemeae. Jour. Botany **66**:171, 172. 1928.

[8] GOSSWEILER, J., Plants from Angola and Portuguese Congo. Jour. Botany Suppl **66**:121–136. 1928.

[9] PITTIER, H., Studies of Venezuelan Bignoniaceae. III. Jour. Wash. Acad. Sci. **18**:333–343. 1928.

[10] FERNALD, M. L., *Geocaulon*, a new genus of the Santalaceae. Contrib. Gray Herb. 79. pp. 21–24. 1928.

[11] ROBINSON, B. L., Records preliminary to a general treatment of the Eupatorieae. Contrib. Gray Herb. 80. pp. 1–42. 1928.

[12] JOHNSTON, I. M., Studies in the Boraginaceae. VII. Contrib. Gray Herb. 81. pp. 3–83. 1928.

[13] ———, Some undescribed American Spermatophytes. Contrib. Gray Herb. 88. pp. 85–98. 1928.

STANDLEY[14] has published 15 new species of Rubiaceae from Central America, representing 4 genera. Most of them belong to *Psychotria* and *Palicourea*, each of which contains 6 of the species.

BROWN,[15] in continuation of his studies of *Mesembryanthemum* and allied genera, has described a new genus (*Dicrocaulon*) which includes 6 species.

TIFFANY[16] has published a very full and well organized account of *Bulbochaete* (Oedogoniaceae), which will be a valuable reference paper for students of algae. He recognizes 51 species, 15 varieties, and 7 forms. One new species and one new variety are described, and the 10 plates make visible the characters used.

BUSH[17] has described as a new species (*Quercus neo-ashei*) the Black Jack oak occurring in middle Texas, which heretofore has been included in *Q. marilandica*.

ETHEL M. DOIDGE[18] has published a very detailed "preliminary account" of the rusts of South Africa, in a contribution from the National Herbarium at Pretoria. It includes descriptions, mostly illustrated, of nearly 400 species, many of which are new, included in 23 genera. The large genera are as follows: *Puccinia* (133 species, 29 new), *Aecidium* (72 species, 16 new), *Uromyces* (71 species, 12 new), *Uredo* (30 species, 5 new), and *Ravenellia* (21 species, 5 new).

Miss DOIDGE, in a subsequent contribution,[19] has described 14 new species of South African Ascomycetes, including a new genus (*Stigmatopeltis*).

STENT,[20] in connection with this study of the South African Gramineae, has described 28 species of *Sporobolus*, 10 of which are new.

PHILLIPS[21] has described the following new genera from South Africa: *Pseudoscolopia* (Flacourtiaceae), *Keetia* (Rubiaceae), and *Theilera* (Campanulaceae).—J. M. C.

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[14] STANDLEY, PAUL C., New plants from Central America. XIII. Jour. Wash. Acad. Sci. **18**:273–282. 1928.

[15] BROWN, N. E., *Mesembryanthemum* and allied genera. Jour. Botany **66**:138–145. 1928.

[16] TIFFANY, L. H., The algal genus *Bulbochaete*. Trans. Amer. Micr. Soc. **47**:131–177. 1928.

[17] BUSH, B. F., *Quercus neo-Ashei*. Bull. Torr. Bot. Club **55**:247–250. 1928.

[18] DOIDGE, ETHEL M., A preliminary study of the South African rust fungi. Bothalia **2**: 1–228. 1926.

[19] ———, South African Ascomycetes in the National Herbarium. Bothalia **2**:229–241. 1927.

[20] STENT, S. M., South African species of *Sporobolus*. Bothalia **2**:247–274. 1927.

[21] PHILLIPS, E. P., Description of three new South African plants. Bothalia **2**:368, 369. 1927.

GENERAL INDEX

Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold-face** type; synonyms in *italics*.

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